REMARKS

Claims 1, 2 and 40-43 are all the claims pending in the application. New claims 40-43 correspond to amended claims 3, 4, 5 and 7, canceled in that last response.

Accordingly, no new matter is added.

Along with the next communication, the Examiner is requested to return signed and initialed copies of the PTO Forms SB/08 that accompanied the Information Disclosure Statements filed May 21, 2009 and herewith.

Claim Rejections - 35 U.S.C. § 103

Claims 1 and 2 are again rejected under 35 U.S.C. 103(a) as being unpatentable over Nishimura et al. (US Patent 6,410,576, hereinafter Nishimura '576) in view of Tsuchiya et al. (European Patent Application EP 1 249 450), Iimura et al. (AJH, 1995), Ishihara et al. (W001/12226) and Nishimura et al. (WO 01/32621, hereinafter Nishimura WO).

The Examiner cites Nishimura '576 as teaching that compounds that exhibit chymase inhibitory activity are "expected to be effective at treating diabetes complications" (columns 9, line 62 to column 10, line 17 and column 52, lines 17-22). The Examiner acknowledges that Nishimura '576 do not teach the specific diabetes complications recited in, for example, claims 41 and 42, or the underlying mechanism creating the complications, as recited in claims 1 and 2. In addition, the Examiner acknowledges that Nishimura '576 does not identify 4-(1-((4-methylbenzo[b]thiophen-3-yl)methyl)benzimidazol-2y1thio)butanoic acid as a chymase inhibitor, as now recited in claim 1 and upon which all pending claims ultimately depend.

Tscuhiya et al. is cited as teaching benzimidazole derivatives that inhibit human chymase and that are clinically applicable as a treating agent for various diseases associated with human

chymase (page 2, paragraphs 1 and 7 and page 67, paragraph 238). The Examiner asserts that Tsuchiya et al. teach that the benzimidizole derivatives have an extremely high chymase inhibitor activity and that one such disclosed derivative is 4-(1-((4-methylbenzo[b]thiophen-3-yl)methyl)benzimidazol-2y1thio)butanoic acid (page 2, paragraph 5 and Page 8, compound 56 and Example 15, page 64).

The Examiner concludes that one of ordinary skill in the art would readily substitute the chymase inhibiting benzimidazole derivatives of Tsuchiya et al. for the chymase inhibitors of Nishimura '576 and expect that the compounds would treat diabetes complications such as glucose intolerance. As to whether a compound that treats complications of diabetes also treats the cause of diabetes, the Examiner states that "in treating the complications associated with diabetes, one would also inherently treat the underlying cause of diabetes which would be the insulin resistance and associated glucose intolerance." Office Action, page 6, para. 21. The Examiner further states that a chemical composition and its properties are inseparable and that, therefore, if the prior art teaches 4-(1-((4-methylbenzo[b]thiophen-3-yl)methyl)benzimidazol-2y1thio)butanoic acid as recited in claim 1, the properties applicant claims are necessarily present.

Iimura et al. is cited as teaching that an ACE inhibitor improves insulin-resistant glucose uptake (insulin sensitivity) in an insulin resistant hypertensive rat model and essential hypertensives, i.e., the subject matter of claim 43.

Ishihara et al. is cited as teaching that compounds having chymase inhibitory effect are expected to be a treatment of diseases such as diabetic retinopathy, as recited in claim 42.

Nishimura 1 is cited as teaching chymase compounds that exhibit excellent inhibitory activity are useful as therapeutic drugs to treat complications of diabetes and obesity, as recited in claim 41.

In response to this rejection in the previous Office action, Applicants asserted that the Examiner's rejection was being made in hindsight. That is, only by reference to the present specification can the Examiner state with certainty that chymase inhibitors, taught as "expected to be effective at treating diabetes complications" would in fact treat the underlying cause of diabetes, because neither reference teaches a relationship between chymase inhibition and the cause of diabetes, i.e., glucose metabolism. The Examiner replies by asserting that since complications of diabetes include impaired glucose tolerance, any compound taught to treat the complications of diabetes would be expected to treat the cause of diabetes as well.

For the following reasons, the rejection is traversed, respectfully.

Nishimura '576 discloses novel thiazolidine derivatives that have chymase inhibitory activity. Further, Nishimura '576 asserts that because the compounds have chymase inhibitory activity they can be expected to be useful as drugs for treating various diseases "originating from chymase," including "outbreaks of physiologic functions such as cardiovascular lesion, inflammation, immune functions and tissue remodeling," as well as "outbreaks of cardiac infarction, heart failure, blood-vessel restenosis after PTCA and the like, [] hypertension [], diabetes complication [], allergic diseases [], asthma [] and the like." Column 9, line 67 - column 10, line 18 and column 52, lines 17-22. Thus, Nishimura '576's disclosure is illustrative in nature and treating diabetes complications is merely one of many theoretically possible, but far from proven, applications of Nishimura '576's compounds. Furthermore, Nishimura '576

does not teach what role chymase inhibitors or Nishimura '576's thiazolidine compounds play in each of the many and varied complications of diabetes. For this reason and in view of the state of the art, Applicants submit that if at the time of the present invention, one of ordinary skill in the art would have expected Nishimura '576's compounds to treat complications of diabetes, it would have been because the compounds would be expected to have PPAR-γ agonistic activity and not because the compounds had chymase inhibitory action. In this respect, the Examiner's attention is directed to the following references:

T. Fujita et al., Reduction of insulin resistance in obese and/or diabetic animals by 5-[4-(1-methylcyclohexylmethoxy)benzyl]-thiazolidine-2,4-dione (ADD-3878, U-63,287, ciglitazone), a new antidiabetic agent. Diabetes, 1983 Sept;32(9);804-810.

B. Hulin et al., Novel thiazolidine-2,4-diones as potent euglycemic agents. J. Med. Chem. 1992 May 15;35(10);1853-64.

RK Vikramadithyan et al., Euglycemic and hypolipidemic activity of PAT5A: a unique thiazolidinedione with weak peroxisome proliferators activated receptor gamma activity. Metabolism. 2000 Nov;49(11):1417-23.

The cited references establish that at the time of the invention, it was well known that thiazolidine derivatives were useful to treat diabetes due to their PPAR-γ agonistic activity. (In fact, Applicants believe that thiazolidine derivatives, such as those sold under the trade names "Pioglitazone" and "ACTOS," are still used to treat diabetes.) Therefore, one of ordinary skill in the art at the time of the invention would generally think that the thiazolidine derivatives in Nishimura '576 possess PPAR-γ activating function, and for that reason, are anti-diabetics. One of ordinary skill in the art would not have expected the chymase inhibitor activity of Nishimura '576's compounds to be relevant to treatment of diabetes or its complications. Thus, one of

ordinary skill in the art wanting to treat diabetes or its complications would not have been motivated to substitute a chymase inhibitor for the thiazolidine derivatives of Nishimura '576.

The secondary and tertiary references also do not provide the needed motivation to replace the thiazolidine derivatives with chymase inhibitors.

Iimura et al (AJH, 1995) reports that an ACE inhibitor and an angiotensin II receptor antagonist improved insulin resistance in an insulin-resistant hypertensive rat model (fructose-fed rats). However, chymase has various actions, in addition to conversion of angiotensin (Ang)I into AngII, such as enhancement of degranulation in mast cells, activation of interleukin-lβ (IL-1β), activation of matrix protease, degradation of fibronectins and type IV collagen, enhancement of release of transforming growth factor-β (TGF-β), activation of substance P and vasoactive intestinal polypeptide (VIP), conversion of endothelin and the like. Therefore, any relationship between the effects of improving glucose intolerance, diabetes, diabetes complications and the like and chymase were difficult to predict. Accordingly, Iimura et al. would not motivate one of ordinary skill in the art to treat diabetes or its complications with a chymase inhibitor.

Furthermore, although Ishihara et al., in the example, shows that compounds having chymase inhibitory activity have angiogenesis inhibiting activity in chorioallantois of chickens, the result relates to only one aspect of a multitude of chymase effects. Ishihara et al. does not report that chymase inhibitors improve glucose intolerance or are useful to treat diabetes or complications thereof. Accordingly, Ishihara et al. would not motivate one of ordinary skill in the art to treat diabetes or its complications with a chymase inhibitor.

Nishimura WO mentions that novel indole derivatives having a chymase inhibitory effect have promise for treating or preventing diabetes complications or obesity. However, Nishimura

WO provides no evidence to support this supposition. Accordingly, Nishimura WO would not motivate one of ordinary skill in the art to treat diabetes or its complications with a chymase inhibitor.

It is clear from the above remarks and cited references, that none of Nishimura '576, limura, Ishihara, and Nishimura WO would have motivated one of ordinary skill in the art to treat diabetes or its complications with a chymase inhibitor. Thus, the fact that the 4-(1-((4-methylbenzo[b]thiophen-3-yl)methyl)benzimidazol-2y1thio)butanoic acid of Tsuchiya et al. inherently has anti-diabetic properties is irrelevant.

It remains that the present invention proved for the first time that chymase inhibitors possess activity to improve glucose intolerance. Further, the present invention used a transgenic mouse (TGM) that produced human chymase. Thus, the credibility of the present application is completely different from the credibility of the cited references.

In view of the above, reconsideration and allowance of this application are now believed to be in order, and such actions are hereby solicited. If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned at the telephone number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,

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Novel Thiazolidine-2,4-diones as Potent Euglycemic Agents

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A new series of thiazolidine-2,4-diones was obtained by replacing the ether function of englitazone with various functional groups, i.e., a ketone, alcohol, or olefin moiety. These compounds lower blood glucose levels in the genetically obese and insulin-resistant ob/ob mouse. Appending an oxazole-based group at the terminus of the chain provided highly potent compounds.

Non-insulin-dependent diabetes mellitus (NIDDM) is a metabolic disorder characterized by hyperglycemia as well as insulin resistance and/or impaired insulin secretion.[1] In the United States 6 million people are diagnosed as non-insulin-dependent diabetics[2] and it has been estimated that an equal number of diabetics remain undiagnosed.[3] A large body of evidence from epidemiological[4] and clinical[5] studies points to a positive relationship between hyperglycemia and long-term organ complications such as neuropathy, nephropathy, retinopathy, and premature atherosclerosis and to the necessity for tight control of blood glucose levels in the early stages of the disease.[6]

Therapy for NIDDM has been aimed at improving glycemic control via a combination of diet, exercise, and oral agents.[7] The most commonly used agents are the sulfonylureas.[8] These compounds stimulate the secretion of insulin in response to glucose and may have extrapancreatic effects as well.[9] Because of the relatively high rate of primary and secondary failure and the high incidence of life-threatening hypoglycemic episodes,[10] new agents which do not stimulate insulin release are being investigated.[11]

In 1982, Takeda, Inc., reported a series of 5-substituted-2,4-thiazolidinediones as a new class of glucose-lowering agents.[12] In this and subsequent papers they showed that these compounds and in particular the prototype ciglitazone lower plasma glucose in animal models of NIDDM but not in nondiabetic animals.[13] Since that time, numerous reports have appeared reflecting efforts to discover more potent and better tolerated members of this class.[14]

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(3) Bagley, J. L. Many Diabetics are Undiagnosed. *Ann. Drug.* 1987, *196*, 68.

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(6) Jaspan, J. B. Monitoring and Controlling the Patient with Non-Insulin Dependent Diabetes Mellitus. *Metabolism* 1987, *36* (Suppl. 1), 22-27.

(7) Gerich, J. E. Oral Hypoglycemic Agents. *N. Engl. J. Med.* 1989, *321*, 1231-1245.

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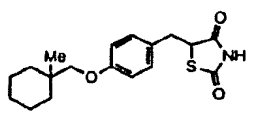
(12) Sohda, T.; Mizuno, K.; Imamiya, E.; Sugiyama, Y.; Fujita, T.; Kawamatsu, Y. Studies on Antidiabetic Agents. II. Synthesis of 5-[4-(1-Methylcyclohexylmethoxy)benzyl]thiazolidine-2,4-dione (ADD-3878) and Its Derivatives. *Chem. Pharm. Bull.* 1982, *30*, 3580-3600.

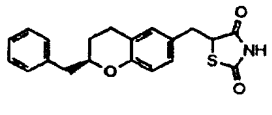
(13) (a) Fujita, T.; Sugiyama, Y.; Taketomi, S.; Sohda, T.; Kawamatsu, Y.; Iwatsuka, H.; Suzuoki, Z. Reduction of Insulin Resistance in Obese and Diabetic Animals by 5-[4-(1-Methylcyclohexylmethoxy)benzyl]thiazolidine-2,4-dione (ADD 3878, U-63,287, Ciglitazone), a New Antidiabetic Agent. *Diabetes* 1983, *32*, 804-810. (b) Chang, A. Y.; Wyse, B. M.; Gilchrist, B. J.; Peterson, T.; Diani, A. R. Ciglitazone, a New Hypoglycemic Agent. I. Studies in ob/ob and db/db Mice, Diabetic Chinese Hamsters, and Normal and Streptozotocin-Diabetic Rats. *Diabetes* 1983, *32*, 830-838. (c) Baba, S.; Doi, K.; Matsuura, M.; Kawara, A.; Tanaka, T.; Ooe, M. New Antidiabetic Agent ADD-3878. *Diabetes* 1982, *31* (Suppl. 2), 77A (302). (d) Sohda, T.; Mizuno, K.; Kawamatsu, Y. Studies on Antidiabetic Agents. VI. Asymmetric Transformation of (±)-5-[4-(1-Methylcyclohexylmethoxy)benzyl]thiazolidine-2,4-dione (Ciglitazone) with Optically Active 1-Phenylethylamines. *Chem. Pharm. Bull.* 1984, *32*, 4460-4465.

0022-2623/92/1835-1853$03.00/0

Our laboratories have recently disclosed a series of dihydrobenzopyran and dihydrobenzofuran thiazolidinediones, including englitazone, a compound undergoing clinical studies,[15] as well as a series of 5-benzyl-2,4-oxazolidinediones.[16] Here we report some studies aimed at the discovery of a second generation of 2,4-thiazolidinedione euglycemics with novel structural features and greatly improved potency.



Ciglitazone

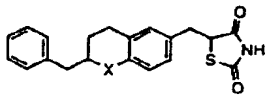


Englitazone

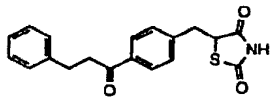
Biological Procedure

Measure of in Vivo Euglycemic Activity. The method used has been described previously[15,16] and is herein repeated for convenience. Six- to eight-week-old C57 BL/6J-ob/ob mice (obtained from Jackson Laboratories, Bar Harbor, ME) were housed five per cage under standard animal care practices. After a 1-week acclimation period, the animals were weighed and 25 μL of blood were collected via the retro-orbital sinus prior to any treatment. The blood sample was immediately diluted 1:5 with saline containing 2% sodium heparin and held on ice for glucose analysis. Animals were then dosed daily for 4 days with drug or vehicle. All drugs were administered by oral gavage, once daily, in a vehicle consisting of 0.25% (w/v) methylcellulose in water with no pH adjustment (0.1 mL of solution per 20 g of animal weight). Animals were bled 24 h after the fourth administration of drug or vehicle (via the retro-orbital sinus) for blood glucose levels. The weight of each animal was recorded on days 1 and 5 of the treatment. The freshly collected samples (125 μL in 330-μL tubes) were centrifuged for 2 min at 10000*g* at room temperature. A 50-μL sample was analyzed for glucose by the Abbott VP Super System Analyzer,[17] using the A-gent[17] glucose UV reagent system[18] (hexokinase method using 100, 300, and 500 mg/dL standards). Ciglitazone was dosed at 50 mg/kg as a positive control and results are reported in all tables as the percentage of glucose normalization compared to the standard ciglitazone-treated group (100% at 50 mg/kg) and the vehicle treated group (0%).

Table I. Euglycemic Activity of Ketones vs Ethers



1 X = O
2 X = CO



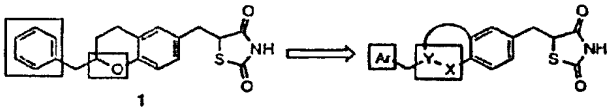
3

compd	% glucose normalization in ob/ob mouse (mg/kg)[a] 25	10	5
1[15]	91[b]	70[b]	48[b]
2	47[b]	54	39[b]
3	93[b]	81[c]	53[d]

[a] Normalization (100%) = ciglitazone effect at 50 mg/kg. [b] $p < 0.05$ when compared to vehicle control. [c] Average of 4 separate tests, ±20%. [d] Average of 2 separate tests, ±15%.

Results and Discussion

Our strategy for the discovery of novel potent euglycemics involved the search for a new sterically and biologically equivalent functional entity to replace the ether function found in ciglitazone and englitazone. It was thought that such an ether bioisostere[19] would allow us entry into a structurally novel class of compounds, while modification of the lipophilic "tail" part of englitazone might greatly improve the potency, as had been the case in the ether/thiazolidinedione[14d] and ether/oxazolidinedione[16] series of compounds.



1

The ether oxygen of the 2-benzylbenzopyran 1[14h,15] was replaced by sulfur, substituted nitrogen, and carbonyl groups. While the sulfur and nitrogen analogues were somewhat inferior in potency,[20] the analogous ketone 2 as well as the alicyclic ketone 3 possessed equivalent activity (Table I).

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(16) Dow, R. L.; Bechle, B. M.; Chou, T. T.; Clark, D. A.; Hulin, B.; Stevenson, R. W. Benzyloxazolidine-2,4-diones as Potent Hypoglycemic Agents. *J. Med. Chem.* 1991, *34*, 1538–1544.

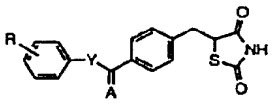
(17) A registered trademark of Abbott Laboratories, Diagnostic Division. 820 Mission Street, So. Pasadena, CA 91030.

(18) A modification of the method of: Richterich, R.; Dauwalder, H. Assay of Blood-Glucose by Hexokinase/Glucose-6-Phosphate Dehydrogenase Method. *Schweiz. Med. Wochenscr.* 1971, *101*, 615.

(19) Lipinski, C. A. Bioisosterism in Drug Design. *Ann. Rep. Med. Chem.* 1986, *21*, 283–291.

(20) Goldstein, S. W.; et al. Manuscript in preparation.

Table II. Euglycemic Activity of Ketone Analogues of 3



compd				% glucose normalization in ob/ob mouse (mg/kg)[a]		
no.	R	A	Y	25	10	5
3	H	O	$(CH_2)_2$	93[b]	81[c]	53[d]
4	H	O	CH_2		0	
5	H	O	$(CH_2)_3$		26	
6	H	O	OCH_2		7	
7	4-OBn	O	$(CH_2)_2$		45[c]	
8	4-Ph	O	$(CH_2)_2$		71[b]	54
9	2-OMe	O	$(CH_2)_2$		0	
10	4-OMe	O	$(CH_2)_2$		50	
11	H	O	CH=CH (*E*)	99[b]	65[b]	23
12	4-OBn	O	CH=CH (*E*)	96[b]	57[b]	64[b]
13	2-OMe	O	CH=CH (*E*)		66[f]	50
14	2-Cl	O	CH=CH (*E*)		44[b]	65[b]
15	2-CF_3	O	CH=CH (*E*)		57[b]	39
16	2-Bn	O	CH=CH (*E*)		56[b]	
17	3-Cl	O	CH=CH (*E*)		4	
18	4-Br	O	CH=CH (*E*)		28	
19	4-COOEt	O	CH=CH (*E*)		4	
20	4-Ph	O	CH=CH (*E*)	65[g]	56	
21	2-OH	O	CH=CH (*E*)	0		
22	2-Me	O	CH=CH (*E*)		21	
23	4-CH_2OMe	O	CH=CH (*E*)	0		
24	4-OMe	O	CH=CH (*E*)		68[b]	0
25	4-NMe_2	O	CH=CH (*E*)		17	
26	H	H, OH	$(CH_2)_2$		89[h]	47[i]
27	H	H_2	$(CH_2)_2$	73[j]	77[k]	46

[a] Normalization (100%) = ciglitazone effect at 50 mg/kg. [b] $p < 0.05$ when compared to vehicle control. [c] Average of 4 separate tests, ± 20%. [d] Average of 2 separate tests, ±16%. [e] Average of 2 separate tests, ±13%. [f] Average of 2 separate tests, ±4%. [g] Average of 2 separate tests, ±6%. [h] Average of 2 separate tests, ±12%. [i] Average of 3 separate tests, ±47%. [j] Average of 2 separate tests, ±14%. [k] Average of 2 separate tests, ±23%.

It is also apparent that the tetralone 2 offers no potency advantage over the open-chain ketone 3. In addition, in the benzopyran series, the stereogenicity at C-2 of the benzopyran ring of 1 has been shown to have little influence over its euglycemic potency.[15] Assuming the same lack of effect of C-2 in the tetralone 2, the cyclic structure would present no distinct advantage over the simpler alicyclic analogue 3. We therefore chose 3 as our new starting point. Close-in analogues of 3 were prepared in order to probe the following areas of the molecule: (a) the pendant phenyl ring; (b) the ethane linker; (c) the ketone function; (d) the "internal" phenyl ring. The results are shown in Table II.

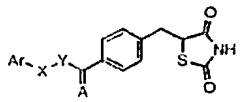
While several compounds generated in this analogue program have potency equivalent to 3, no significant improvement was achieved. The ethane linker could not be shortened (4), lengthened (5), or incorporate an oxygen (6) without virtually complete loss of activity. Introduction of a double bond in this spacer, however, produced compounds with similar activity (11, 12). Attempting to increase the potency by substitution on the pendant phenyl ring proved equally difficult and only equipotent compounds to 3 could be found (7–25). Manipulation of the carbonyl group of 3 gave interesting results. While the activity of the alcohol 26 (equivalent to 3) was not unexpected, to our surprise, the methylene analogue 27 was also equipotent, indicating that the presence of an oxygen functionality, or indeed of any functionality, is not required at that position.

Our next step was to replace the phenyl tail by a heterocycle. The initial results are presented in Table III.

As was the case in the ether/thiazolidinedione[14e] and ether/oxazolidinedione[16] series, replacement of the phenyl group by a 4-linked oxazole leads to a remarkable jump in potency, as exemplified by 32 (CP-86,325). This effect appears to be specific to the 4-oxazole moiety: the analogous thiazole group displayed considerably weaker activity. In addition, introduction of a furan (28, 31), pyridine (33, 34), or benzothiazole (29) ring at the end of the ethane linker results in similar to or markedly lower activity than that of 3. We thus proceeded to explore the structure–activity relationship (SAR) around 32, further introducing fine modifications in all areas of the molecule except the oxazole ring which appears to be essential to ensure high potency. Table IV illustrates the influence of the substitution on the oxazole ring. While removal of the 5-methyl group lowers the activity, the 2-position is amenable to substitution. Indeed, the phenyl ring can be substituted with a 4-trifluoromethyl (41) or 3,5-dimethyl-4-methoxy (44) group, or replaced by a 4-methyl-2-furanyl group (47) with retention of the activity. These four compounds (32, 41, 44, 47) have an ED_{50} (defined as the dose causing 50% normalization) close to or below 0.1 mg/kg, a considerable improvement over 3 and 1 (ED_{50} ca. 5 mg/kg).

Table V shows the activity of various reduction products

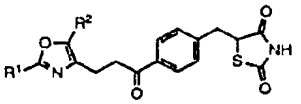
Table III. Euglycemic Activity of Ketones and Alcohols Equipped with Heterocyclic Tails



compd				% glucose normalization in ob/ob mouse (mg/kg)[a]		
no.	A	Ar	X–Y	10	5	2.5
28	O	2-furyl	CH=CH (*E*)	65[b]		
29	O	2-benzothiazolyl	CH=CH (*E*)	0		
30	O	5-methyl-2-phenyl-4-oxazolyl	CH=CH (*E*)	59	100[b]	
31	O	2-furyl	CH_2—CH_2	24		
32	O	5-methyl-2-phenyl-4-oxazolyl	CH_2—CH_2	100[b]	100[b]	88[c]
33	H, OH	2-pyridyl	CH_2—CH_2	13		
34	O	5-ethyl-2-pyridyl	CH_2—CH_2	41	0	
35	O	2-phenyl-4-thiazolyl	CH_2—CH_2		91[b]	48[b]
36	O	4-methyl-2-phenyl-5-thiazolyl	CH_2—CH_2		75[b]	33[d]
37	O	2-methyl-4-phenyl-5-thiazolyl	CH_2—CH_2		61[b]	0

[a] Normalization (100%) = ciglitazone effect at 50 mg/kg. [b] $p < 0.05$ when compared to vehicle control. [c] Average of 2 tests, ±12%. [d] Average of 2 tests, ±1%.

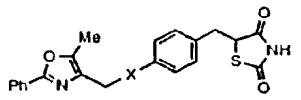
Table IV. Effect of Substitution of the Oxazole Ring on Euglycemic Activity



compd			% glucose normalization in ob/ob mouse (mg/kg)[a]			
no.	R^1	R^2	1	0.5	0.25	0.1
32	Ph	Me	100[b]	97[c]	88[d]	83[c]
38	Ph	H		30	52[f]	
39	4-OMe-Ph	Me	95[b]	43[b]		
40	4-Me-Ph	Me	47[e]	47[b]	15[f]	
41	4-CF_3-Ph	Me	86[b]	69[b]	81[b]	88[b]
42	4-Me-Ph	H		0	15[f]	
43	4-OH-Ph	Me	0			
44	4-OMe-3,5-Me_2-Ph	Me	100[b]	100[b]		75[b]
45	4-OH-3,5-Me_2-Ph	Me	100[b]	30		
46	2-furyl	Me	61[b]			
47	2-(5-methylfuryl)	Me		68[b]	87[b]	47
48	2-thienyl	Me	84[b]	71[b]	27	
49	2-naphthyl	Me	39[k]			

[a] Normalization (100%) = ciglitazone effect at 50 mg/kg. [b] $p <$ 0.05 when compared to vehicle control. [c] Average of 3 separate tests, ±5%. [d] Average of 3 separate tests, ±13%. [e] Average of 5 separate tests, ±30%. [f] Average of 2 separate tests, ±3%. [g] Average of 3 separate tests, ±31%. [h] Average of 2 separate tests, ±32%. [i] Average of 2 separate tests, ±1%. [j] Average of 2 separate tests, ±10%. [k] Average of 2 separate tests, ±20%.

Table V. Euglycemic Activity of Ketone Reduction Products



compd		% glucose normalization in ob/ob mouse (mg/kg)[a]			
no.	X	0.5	0.25	0.1	0.05
50	CH_2CHOH	100[b]	85[c]	93[b]	72[b]
51	CH=CH	84[d]	74[e]	29[b]	
52	$(CH_2)_2$	37			

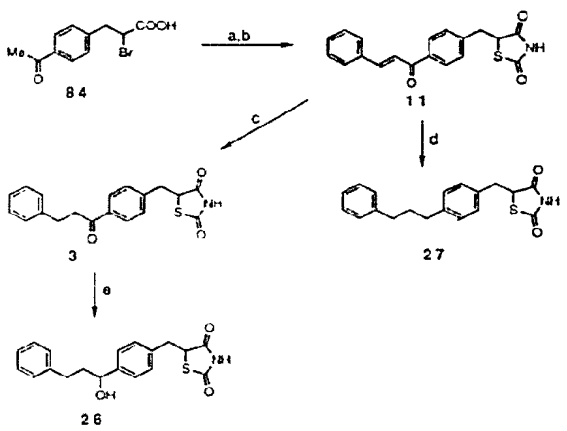
[a] Normalization (100%) = ciglitazone effect at 50 mg/kg. [b] $p <$ 0.05 when compared to vehicle control. [c] Average of 4 separate tests, ±12%. [d] Average of 2 separate tests, ±8%. [e] Average of 2 separate tests, ±3%.

of the ketone function of **32**. The alcohol is fully active, the olefin **51** is also very potent although somewhat less so than **32**, and the hydrocarbon **52** is clearly inferior. Changing the central phenyl ring into a 2,4-disubstituted thiophene led to compounds generally less active (Table VI). Interestingly, in this group of compounds the olefin **59** was more potent than the corresponding ketone **57**, a reversal of the potency order seen between **32** and its corresponding olefin **51**.

With the ED_{50} comfortably below 0.5 mg/kg, we proceeded to search for new isosteres of the ketone function in order to expand the scope of this study. As shown in Table VII, all sulfides, sulfones, amides, and sulfonamides examined were comparatively inactive. The oxime **72** showed a 10-fold drop in potency. It is unclear whether this compound is converted in vivo to the ketone **32**.[21]

(21) See, for example: (a) Sternson, L. A. Species Variation in the Metabolism of Acetophenone Oxime by Hepatic Enzymes. *Pharmacology* **1975**, *13*, 234. (b) Hucker, B.; Michniewic, B. M.; Rhodes, R. E. Phenylacetone Oxime–An Intermediate in the Oxidative Deamination of Amphetamine. *Biochem. Pharmacol.* **1971**, *20*, 2123–2128.

Scheme I[a]



[a] (a) Thiourea, sulfolane, 110 °C, then 2 N HCl, 110 °C; (b) benzaldehyde, NaOMe, ethanol; (c) triethylsilane (1 equiv), trifluoroacetic acid, 0 °C; (d) triethylsilane (3 equiv), trifluoroacetic acid, 0 °C to room temperature; (e) sodium borohydride, methanol.

Finally, Table VIII examines the activity of the α,β-unsaturated thiazolidinediones. The parent compound **75**, as well as the thiophene analogue **82**, appears to have comparable activity to **32**, while all other analogues prepared showed reduced activity at 1 mg/kg. However, the insolubility of these compounds makes any precise analysis difficult.

In summary, we have shown that the ether linkage of englitazone could be adequately replaced by carbonyl, carbinol, or olefin groups and that high potency could be obtained by appending the 5-methyl-2-aryl-4-oxazolyl tail. Based on its remarkable in vivo potency (50–100-fold improvement over englitazone), CP-86,325 (**32**) was selected for clinical studies.

Chemistry

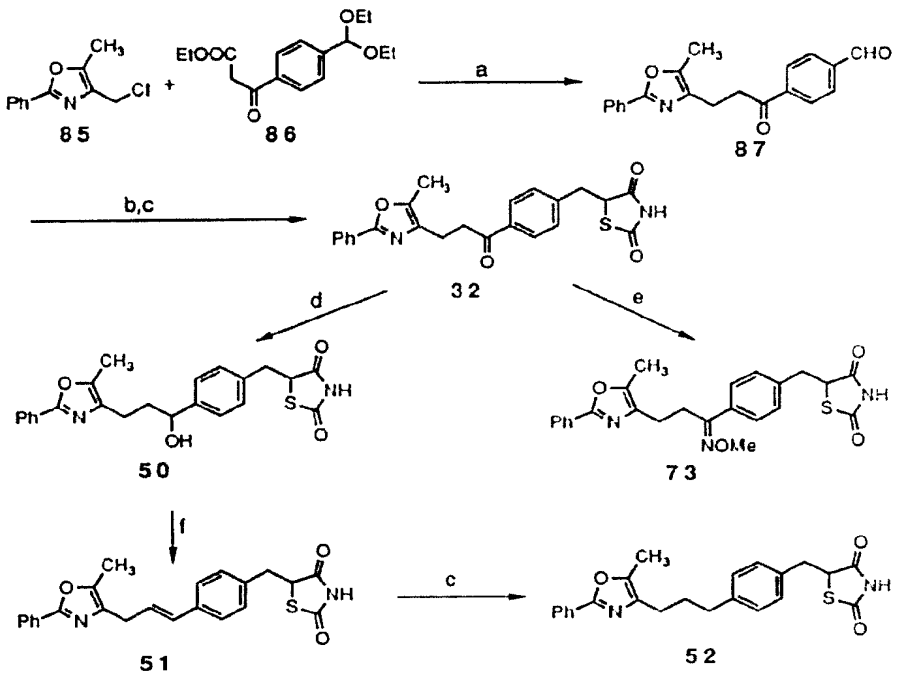
Each compound in this paper was prepared according to one of the standard methods described in the schemes below. Table IX contains analytical data and shows which method was used for each individual compound.

All phenethyl ketones were synthesized by aldol condensation[22] followed by reduction to the saturated ketone, the alcohol, or the alkane (Scheme I). The heterocycle-substituted ethyl ketones were prepared by alkylation of keto ester **86** followed by decarboxylation/deprotection and conversion of the aldehyde to the thiazolidinedione by the standard procedure[15] (Scheme II). The amides and sulfonamides were put together by straightforward coupling reactions (Scheme III).

The shortened-chain and oxygen-inserted compounds **4** and **6** were prepared according to Schemes IV and V, respectively. The sulfides were synthesized by alkylation of 4-bromothiophenol followed by formylation and conversion of the aldehyde to the thiazolidinedione, and the sulfones were obtained by *m*-chloroperbenzoic acid oxidation (Scheme VI). The thiophene compounds were prepared in a similar fashion to the phenyl analogues (Scheme VII), the aldehyde function being introduced at a later stage in the synthesis. All saturated thiazolidinediones are racemic compounds. All secondary alcohols (e.g., **50**) are mixtures of the four possible isomers.

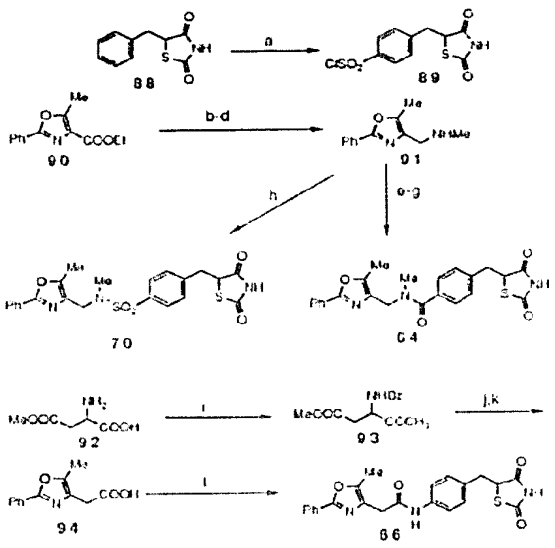
(22) Nielsen, A. T.; Houlihan, W. J. The Aldol Condensation. *Org. React.* **1968**, *16*, 1–144.

Scheme II[a]



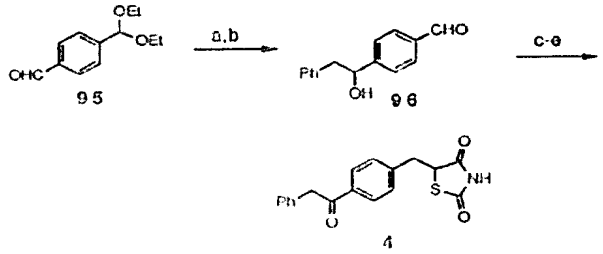
[a] (a) Sodium hydride, THF, then NaOH, then 1 N HCl; (b) 2,4-thiazolidinedione, piperidine, ethanol, reflux; (c) H_2, Pd–C; (d) sodium borohydride, 2-propanol; (e) methoxyamine hydrochloride, pyridine, ethanol; (f) *p*-toluenesulfonic acid, toluene, reflux.

Scheme III[a]



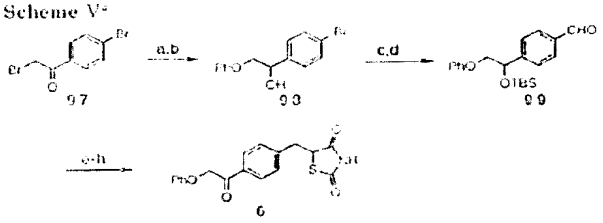
[a] (a) Chlorosulfonic acid; (b) $LiAlH_4$, ether; (c) PDC, CH_2Cl_2; (d) $MeNH_2$, $MgSO_4$, ether then $NaBH_4$, methanol; (e) 4-carboxybenzaldehyde, *i*-BuOCOCl, Et_3N, CH_2Cl_2; (f) 2,4-thiazolidinedione, NaOAc, 140 °C; (g) H_2, Pd–C, THF; (h) 89, *i*-Pr_2EtN, CH_2Cl_2; (i) BzCl, pyridine, then Ac_2O, 90 °C; (j) $POCl_3$, toluene, reflux; (k) 1 N NaOH, reflux; (l) 5-(4-aminobenzyl)thiazolidine-2,4-dione, ClCOOEt, Et_3N, CH_2Cl_2.

Scheme IV[a]



[a] (a) BnMgBr, ether, −30 °C; (b) $HClO_4$, THF; (c) 2,4-thiazolidinedione, NaOAc, 140 °C; (d) Na(Hg), methanol; (e) H_2CrO_4, ether.

Scheme V[a]

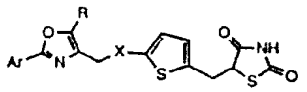


[a] (a) Phenol, K_2CO_3, acetone; (b) $NaBH_4$, *i*-PrOH, CH_2Cl_2; (c) *t*-BuMe$_2$SiCl, imidazole, DMF; (d) *n*-BuLi, DMF, THF; (e) 2,4-thiazolidinedione, NaOAc, 140 °C; (f) Na(Hg), methanol; (g) HCl-O_4, THF; (h) H_2CrO_4, ether.

Experimental Section

Melting points were taken using a Thomas Hoover apparatus and are uncorrected. Elemental analyses were carried out by the Analytical Department of Pfizer Central Research and results obtained for specified elements are within ±0.4% of the theoretical values unless otherwise denoted. ^1H NMR spectra of deuteriochloroform or DMSO-d_6 solutions (internal standard TMS, δ 0 or the solvent was utilized as an internal standard and deuterium lock) were recorded on Varian A-60, Bruker AM-250, or Varian XL-300 spectrometers. Low resolution and high resolution mass spectra were obtained on Finnigan 4510 and AEI MS-30 instruments, respectively. Compounds analyzed by high resolution mass spectroscopy (HRMS) were >95% pure, as determined by proton NMR and thin-layer chromatography. Infrared spectra were recorded on a Perkin-Elmer 283 spectrophotometer.

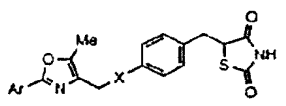
Table VI. Euglycemic Activity of Thiophene Analogues of **32**



compd				% glucose normalization in ob/ob mouse (mg/kg)[a]				
no.	X	R	Ar	5	2.5	1	0.5	0.25
53	CH_2CHOH	Me	Ph	100[b]		23		
54	CH_2CO	Me	Ph	100[b]	99[b]	37		
55	CH_2CO	Me	4-Cl-Ph		100[b]	51[c]		
56	CH_2CO	H	4-Me-Ph		0	10		
57	CH_2CO	Me	4-Me-Ph			100[b]	27	
58	CH_2CO	Me	4-CF_3-Ph			78[c]	27[d]	
59	CH=CH	Me	4-Me-Ph		90[b]	98[c]	73[b]	64[f]

[a] Normalization (100%) = ciglitazone effect at 50 mg/kg. [b] $p < 0.05$ when compared to vehicle control. [c] Average of 2 separate tests, ±22%. [d] Average of 2 separate tests, ±27%. [e] Average of 2 separate tests, ±2%. [f] Average of 2 separate tests, ±3%.

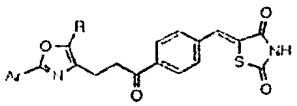
Table VII. Euglycemic Activity of Various Ketone Isosteres



compound			% glucose normalization in ob/ob mouse (mg/kg)[a]			
no.	X	Ar	5	2.5	1	0.5
60	CH_2S	Ph				0
61	CH_2SO_2	Ph	69[b]		11	
62	CH_2SO_2	2-naphthyl	0	0		
63	NHCO	Ph	50[b]	49		
64	NMeCO	Ph	83	0		
65	NPhCO	Ph	23			
66	CONH	Ph	64[b]	16		
67	CONMe	Ph	9			
68	CONEt	Ph	25			
69	$NHSO_2$	Ph	0			
70	$MeNSO_2$	Ph	9			
71	$PhNSO_2$	Ph		32		
72	$ClH_2C(NOH)$	Ph	100[b]		99[c]	0
73	$CH_2C(NOMe)$	Ph	100[b]		0	
74	$CH_2C(NOBn)$	Ph	17			

[a] Normalization (100%) = ciglitazone effect at 50 mg/kg. [b] $p < 0.05$ when compared to vehicle control.

Table VIII. Euglycemic Activity of Unsaturated Thiazolidinediones

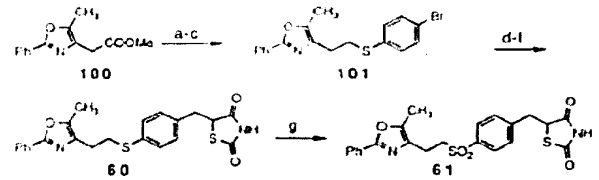


compd			% glucose normalization in ob/ob mouse (mg/kg)[a]		
no.	Ar	R	1	0.5	0.1
75	Ph	Me	84[b]	81[c]	13
76	4-OMe-Ph	Me	62[d]		
77	4-Me-Ph	Me	0	27	
78	4-CF_3-Ph	Me	55[d]	28[c]	
79	4-Me-Ph	H	53[d]		
80	2-furyl	Me	55		
81	2-(5-methylfuryl)	Me	76[b]	1	
82	2-thiophenyl	Me	100[b]		
83	2-naphthyl	Me	90[b]	38[d]	

[a] Normalization (100%) = ciglitazone effect at 50 mg/kg. [b] $p < 0.05$ when compared to vehicle control. [c] Average of 2 separate tests, ±28%. [d] Average of 2 separate tests, ±11%.

5-(4-Acetylbenzyl)thiazolidine-2,4-dione. A mixture of 2-bromo-3-(4-acetylphenyl)propanoic acid[23] (84, 89 g, 0.33 mol), thiourea (50 g, 0.66 mol), and sulfolane (100 mL) was heated to 110 °C for 5 h. Then 2 N HCl (165 mL) was added and the mixture was heated to 110 °C overnight. After cooling, the solution was diluted with ice–water (700 mL), and the solid was collected, washed with water, and dried (77 g, 94%, mp 170.5–171.5 °C): ^{1}H NMR (250 MHz, DMSO-d_6) δ 2.55 (s, 3 H), 3.25 (dd, J = 14, 9 Hz, 1 H), 3.50 (dd, J = 14, 4 Hz, 1 H), 4.95 (dd, J = 9, 4 Hz, 1 H), 7.40 (d, J = 8 Hz, 2 H), 7.85 (d, J = 8 Hz, 2 H), 12.05 (br s, 1 H); ^{13}C NMR (63 MHz, DMSO-d_6) δ 26.6, 36.9, 52.0, 128.2, 129.5, 171.3, 175.4, 222.0.

Scheme VI[a]



[a] (a) $LiAlH_4$, ether; (b) Ph_3P, CBr_4, ether; (c) 4-bromothiophenol, NaH, THF; (d) BuLi, DMF, THF; (e) 2,4-thiazolidinedione, piperidine, ethanol; (f) Na(Hg), methanol; (g) m-CPBA, CH_2Cl_2.

(*E*)-5-[4-(3-Phenyl-2-propenoyl)benzyl]thiazolidine-2,4-dione (11). To a slurry of 5-(4-acetylbenzyl)thiazolidine-2,4-dione (1.0 g, 4.0 mmol) and benzaldehyde (0.41 mL, 4.0 mmol) in ethanol (10 mL) was added sodium methoxide (4.8 mmol, 0.26 g). The solution was heated to reflux for 1.5 h and then cooled, diluted with water (60 mL), acidified with 2 N HCl, and extracted with ethyl acetate (2 × 50 mL). The combined extracts were washed with water (50 mL), dried over sodium sulfate, and concentrated. The residue was recrystallized from 2-propanol to give the title product as a pale yellow solid (0.33 g, 24%, mp 147–149 °C): ^{1}H NMR (250 MHz, $CDCl_3$) δ 3.24 (dd, J = 14.1, 9.5 Hz, 1 H), 3.45 (dd, J = 14.1, 4.0 Hz, 1 H), 4.57 (dd, J = 9.5, 4.0 Hz, 1 H), 7.37 (d, J = 8.3 Hz, 2 H), 7.41–7.43 (m, 3 H), 7.51 (d, J = 15.7 Hz, 1 H), 7.63–7.66 (m, 2 H), 7.82 (d, J = 15.7 Hz, 1 H), 7.98 (d, J = 8.3 Hz, 2 H), 8.71 (br s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 38.5, 52.7, 121.8, 128.5, 129.0, 129.1, 129.6, 130.7, 134.8, 137.6, 145.2, 169.8, 173.7, 190.1; IR (KBr) ν (cm^{-1}) 770, 1155, 1220, 1330, 1570, 1590, 1660, 1710 (s), 1725, 1750, 3060, 3200; MS (EI) m/e 337 (M^+), 221, 193, 178, 131, 118, 103, 90, 77, 51. Anal. ($C_{19}H_{15}NO_3S$) C, H, N.

5-[4-(3-Phenylpropionyl)benzyl]thiazolidine-2,4-dione (3). To an ice-cooled solution of 11 (2.0 g, 5.9 mmol) in trifluoroacetic acid (20 mL) was added triethylsilane (0.95 mL, 5.9 mmol). The mixture was stirred for 25 min at 0 °C and then diluted with water (50 mL) and extracted with ether (2 × 40 mL). The combined extracts were washed with water (2 × 40 mL) and 5% sodium bicarbonate (2 × 40 mL), dried over sodium sulfate, and concentrated. The residue was triturated with hexane to give 3 as a pale yellow solid (1.65 g, 82%, mp 119–121 °C): ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.95 (t, J = 7.5 Hz, 2 H), 3.20 (dd, J = 14, 9

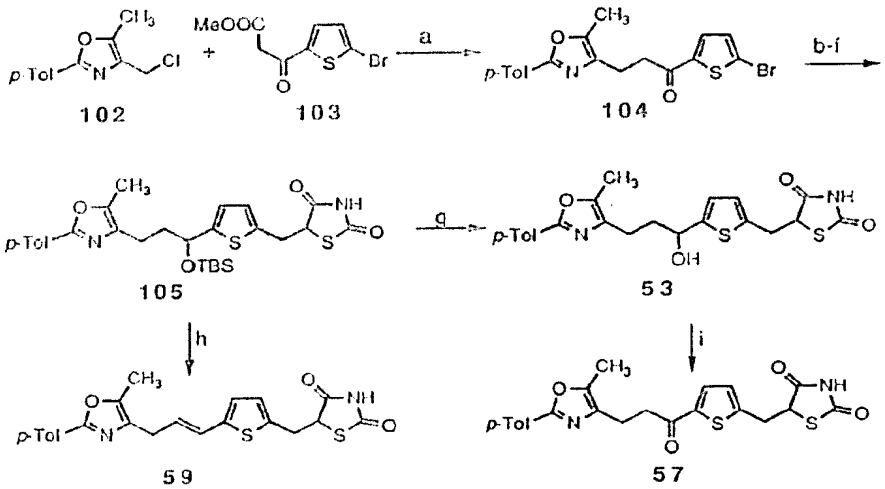
(23) Cleland, G. H. *p*-Acetyl-α-bromohydrocinnamic Acid. *Organic Syntheses*; Wiley: New York, 1988; Collect. Vol. VI, pp 21–23.

Table IX. Analytical Data and Preparation

compd	formula	mp (°C)	prep method[a]	anal.	compd	formula	mp (°C)	prep method[a]	anal.
2	$C_{21}H_{18}NO_3SNa \cdot {}^1/_2H_2O$	288–289	A	C,H,N	**43**	$C_{23}H_{20}N_2O_5S \cdot {}^1/_2H_2O$	218–219	H	C,H,N
3	$C_{19}H_{17}NO_3S$	119–121	A	C,H,N	**44**	$C_{26}H_{22}N_2NaO_5S$	225–235	A	C,H,N
4	$C_{18}H_{15}NO_3S$	145–146	D	C,H,N	**45**	$C_{27}H_{23}N_2NaO_5S \cdot 2H_2O$	150–200	H	C,H,N
5	$C_{20}H_{19}NO_3S \cdot {}^1/_4H_2O$	138–140	A	C,H,N	**46**	$C_{21}H_{18}N_2O_5S \cdot {}^1/_3C_4H_8O_2$	155–156	A	C,H,N
6	$C_{18}H_{15}NO_4S$	170–174	E	C,H,N	**47**	$C_{27}H_{26}N_2NaO_5S$	208–210	A	HRMS
7	$C_{26}H_{23}NO_4S \cdot H_2O$	97–101	B	C,H,N	**48**	$C_{21}H_{18}N_2O_4S_2 \cdot {}^1/_4H_2O$	157–158	A	C,H,N
8	$C_{25}H_{21}NO_3S \cdot {}^1/_2H_2O$	99–100	B	C,H,N	**49**	$C_{27}H_{26}N_2O_4S$	188–189	A	C,H,N
9	$C_{27}H_{19}NNaO_4S$	269 dec	B	C,H,N	**50**	$C_{23}H_{21}N_2NaO_4S$	268–272	B	C,H,N
10	$C_{20}H_{18}NNaO_4S \cdot {}^1/_2H_2O$	272–274	B	C,H,N	**51**	$C_{23}H_{19}N_2NaO_3S \cdot H_2O$	285 dec	B	C,H,N
11	$C_{19}H_{15}NO_3S$	147–149	B	C,H,N	**52**	$C_{25}H_{22}N_2O_3S$	98–99	B	C,H,N
12	$C_{26}H_{21}NO_4S \cdot {}^1/_2H_2O$	240 dec	B	C,H,N	**53**	$C_{21}H_{19}N_2NaO_4S_2$	206–210	G	HRMS
13	$C_{20}H_{17}NO_4S \cdot H_2O$	gum	B	C,H,N	**54**	$C_{21}H_{18}N_2O_4S_2$	164–166	G	C,H,N
14	$C_{19}H_{14}ClNO_3S \cdot {}^1/_4H_2O$	193–196	B	C,H,N	**55**	$C_{21}H_{17}ClN_2O_4S_2$	147–149	G	HRMS
15	$C_{20}H_{13}NNaO_3S \cdot {}^1/_2H_2O$	251–254	B	C,H,N	**56**	$C_{21}H_{18}N_2O_4S_2 \cdot {}^1/_2C_2H_4Cl_2$	151–153	G	C,H,N
16	$C_{26}H_{21}NO_3S \cdot {}^1/_2H_2O$	135–136	B	C,H,N	**57**	$C_{22}H_{20}N_2O_4S_2 \cdot {}^1/_2H_2O$	158–160	G	C,H,N
17	$C_{19}H_{14}ClNO_3S \cdot {}^1/_4H_2O$	162.5–164	B	C,H,N	**58**	$C_{22}H_{17}F_3N_2O_4S_2$	157–159	G	C,H,N
18	$C_{19}H_{13}BrKNO_3S$	>250	B	C,H,N	**59**	$C_{23}H_{19}N_2NaO_4S_2$	245–250	G	C,H,N
19	$C_{22}H_{19}NO_5S \cdot {}^1/_4H_2O$	173	B	C,H,N	**60**	$C_{22}H_{19}N_2NaO_4S_2$	268–270	F	C,H,N
20	$C_{28}H_{19}NO_5S \cdot {}^1/_4H_2O$	198.5–200	B	C,H,N	**61**	$C_{22}H_{20}N_2O_5S_2$	foam	F	HRMS
21	$C_{19}H_{15}NO_4S$	150–153	B	C,H,N	**62**	$C_{27}H_{22}N_2O_5S_2$	77–80	F	HRMS
22	$C_{20}H_{17}NO_5S \cdot H_2O$	115–120	B	C,H,N	**63**	$C_{23}H_{19}N_3O_4S \cdot H_2O$	101–103	C	C,H,N
23	$C_{21}H_{14}NNaO_4S \cdot {}^3/_4H_2O$	282–286	B	C,H,N	**64**	$C_{23}H_{21}N_3O_4S \cdot H_2O$	75–78	C	C,H,N
24	$C_{20}H_{17}NO_4S \cdot {}^1/_4H_2O$	177–180	B	C,H,N	**65**	$C_{28}H_{23}N_3O_4S$	95–98	C	HRMS
25	$C_{21}H_{18}N_2NaO_3S \cdot H_2O$	284–286	B	C,H,N	**66**	$C_{23}H_{19}N_3O_4S \cdot {}^1/_2H_2O$	201–202	C	C,H,N
26	$C_{19}H_{14}NNaO_3S \cdot {}^3/_4H_2O$	249–254	A	C,H,N	**67**	$C_{23}H_{21}N_3O_4S \cdot {}^3/_4H_2O$	75–77	C	C,H,N
27	$C_{19}H_{19}NO_2S$	84–86	A	C,H,N	**68**	$C_{24}H_{23}N_3O_4S \cdot {}^1/_2H_2O$	68.5–70	C	C,H,N
28	$C_{17}H_{12}KNO_4S \cdot {}^1/_4H_2O$	270–275	B	C,H,N	**69**	$C_{21}H_{19}N_3O_3S_2$	120–122	C	HRMS
29	$C_{20}H_{14}N_2O_3S_2 \cdot {}^1/_2H_2O$	200–204	B	C,H,N	**70**	$C_{22}H_{21}N_3O_3S_2 \cdot {}^1/_4H_2O$	83–84	C	C,H,N
30	$C_{23}H_{18}N_2O_4S \cdot {}^1/_4H_2O$	190–192	B	C,H,N	**71**	$C_{28}H_{25}N_3O_3S_2$	68–71	C	C,H,N
31	$C_{17}H_{15}NO_4SNa \cdot {}^1/_4H_2O$	265–270	B	C,H,N	**72**	$C_{23}H_{21}N_3O_4S$	202–205	B	C,H,N
32	$C_{25}H_{20}N_2O_4S$	145–146	B	C,H,N	**73**	$C_{24}H_{23}N_3O_4S \cdot {}^1/_2H_2O$	138–140	B	C,H,N
33	$C_{18}H_{17}N_2NaO_2S \cdot {}^3/_2H_2O$	125–130	A	C,H,N	**74**	$C_{30}H_{27}N_3O_4S$	175–177	B	C,H,N
34	$C_{20}H_{20}N_2O_2S$	168–169	A	C,H,N	**75**	$C_{22}H_{18}N_2O_4S$	224–225	B	C,H,N
35	$C_{22}H_{18}N_2O_2S_2$	139–142	A	C,H,N	**76**	$C_{24}H_{20}N_2O_5S$	223–224	A	C,H,N
36	$C_{23}H_{20}N_2O_2S_2$	118–120	A	C,H,N	**77**	$C_{24}H_{22}N_2O_4S$	250–251	A	C,H,N
37	$C_{23}H_{19}N_2NaO_2S_2$	210–220 dec	A	C,H,N	**78**	$C_{24}H_{17}F_3N_2O_4S$	244–245	A	C,H,N
38	$C_{22}H_{18}N_2O_2S_2$	151–155	A	C,H,N	**79**	$C_{25}H_{18}N_2O_4S$	220–223	A	C,H,N
39	$C_{24}H_{22}N_2O_2S$	173–174	A	C,H,N	**80**	$C_{21}H_{16}N_2O_4S$	237–238	A	C,H,N
40	$C_{24}H_{22}N_2O_4S$	240–242	A	C,H,N	**81**	$C_{25}H_{18}N_2O_5S \cdot {}^1/_4H_2O$	236–238	A	C,H,N
41	$C_{24}H_{19}F_3N_2O_4S$	244–245	A	C,H,N	**82**	$C_{21}H_{16}N_2O_4S_2$	237–238	A	C,H,N
42	$C_{23}H_{20}N_2O_4S$	143–144	A	C,H,N	**83**	$C_{27}H_{20}N_2O_4S$	221–222	A	C,H,N

[a] Method A is shown in Scheme I; method B is shown in Scheme II; method C is shown in Scheme III; method D is shown in Scheme IV; method E is shown in Scheme V; method F is shown in Scheme VI; method G is shown in Scheme VII; method H, demethylation of **42** or **44** with HBr, AcOH.

Scheme VII[a]



[a] (a) NaH, THF, then NaOH, then HCl; (b) $NaBH_4$, ethanol; (c) *t*-BuMe$_2$SiCl, imidazole, DMF; (d) *n*-BuLi, DMF, THF; (e) 2,4-thiazolidinedione, NaOAc, 140 °C; (f) Na(Hg), methanol; (g) $HClO_4$, THF; (h) 6 N HCl, THF; (i) PDC, CH_2Cl_2.

Hz, 1 H), 3.55 (dd, J = 14, 4 Hz, 1 H), 4.95 (dd, J = 9, 4 Hz, 1 H), 7.15 (m, 1 H), 7.25 (m, 4 H), 7.45 (d, J = 8 Hz, 2 H), 7.95 (d, J = 8 Hz, 2 H), 12.1 (br s, 1 H). Anal. ($C_{19}H_{17}NO_3S$) C, H, N.

5-[4-(3-Phenylpropyl)benzyl]thiazolidine-2,4-dione (27). A solution of 11 (2.0 g, 5.9 mmol) in trifluoroacetic acid (20 mL), cooled to 0 °C, was treated with triethylsilane (2.8 mL, 18 mmol). After 25 min at 0 °C and 25 min at room temperature, the solution was diluted with water (60 mL) and extracted with ether (2 × 50 mL). The combined extracts were washed with water (2 × 50 mL) and brine (50 mL), dried over sodium sulfate, and concentrated, leaving a brown oil. The product was isolated by flash chromatography (hexanes/ethyl acetate, 5:1) as a solid (1.11 g, 58%, mp 84–86 °C): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.03 (quin, J = 7.6 Hz, 2 H), 2.71 (t, J = 6.7 Hz, 2 H), 2.73 (t, J = 6.9 Hz, 2 H), 3.13 (dd, J = 13.9, 10.2 Hz, 1 H), 3.58 (dd, J = 14.0, 3.7 Hz, 1 H), 4.56 (dd, J = 10.0, 3.7 Hz, 1 H), 7.22–7.35 (m, 8 H), 7.38 (t, J = 7.7 Hz, 1 H), 9.61 (br s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 32.95, 35.14, 35.56, 38.36, 53.83, 125.95, 128.49, 128.59, 129.05, 129.21, 133.46, 141.83, 171.72, 175.42; IR (KBr) ν (cm^{-1}) 1170, 1340, 1700 (s), 1740, 3200 (br). Anal. ($C_{19}H_{19}NO_3S$) C, H, N.

5-[4-(1-Hydroxy-3-phenylpropyl)benzyl]thiazolidine-2,4-dione (26). To an ice-cooled solution of 3 (1.65 g, 4.9 mmol) in methanol (35 mL) was added sodium borohydride (0.19 g, 4.9 mmol). The solution was stirred for 2 h at room temperature and then quenched with 1 N HCl, diluted with water (100 mL), and extracted with ethyl acetate (3 × 50 mL). The combined extracts were washed with water (50 mL) and brine (50 mL), dried over sodium sulfate, and concentrated, leaving 26 as an oil (1.6 g, 97%). The sodium salt was prepared by combining the product (1.6 g, 4.7 mmol) and sodium methoxide (0.26 g, 4.7 mmol) in methanol (15 mL) and concentrating the solution to a yellow solid (mp 249–254 °C): ^{1}H NMR (250 MHz, DMSO-d_6) δ 1.86–1.88 (m, 2 H), 2.55–2.73 (m, 3 H), 3.67 (dd, J = 13.9, 3.3 Hz, 1 H), 4.19 (dd, J = 10.6, 3.4 Hz, 1 H), 4.50 (t, J = 6.1 Hz, 1 H), 7.15–7.28 (m, 9 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 31.62, 31.93, 40.93, 58.68, 71.51, 125.57, 125.69, 128.23, 128.42, 138.48, 142.11, 143.97, 181.59, 190.65; IR (KBr) ν (cm^{-1}) 700, 1240, 1330, 1550, 1570 (s), 1670, 3360; MS (EI) m/e 281, 176, 105, 91, 77, 60. Anal. ($C_{19}H_{18}NNaO_3S\cdot{}^3/_4H_2O$) C, H, N.

1-[4-(Diethoxymethyl)phenyl]ethanol (106). 4-(Diethoxymethyl)benzaldehyde (104 g, 0.5 mol) was dissolved in ether (300 mL) and the resulting solution cooled to −75 °C. With vigorous stirring, methyllithium (390 mL of a 1.4 M ether solution, 0.55 mol) was added at a rate which maintained the temperature below −60 °C. The reaction mixture was allowed to warm to room temperature, poured into ice–water (500 mL), and stirred for 10 min, and the layers were separated. The aqueous layer was extracted with ether (500 mL). The combined organic layers were washed with water (500 mL) and brine (500 mL), dried over magnesium sulfate, and concentrated to yield a viscous yellow oil (110 g, 98%): ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.2 (t, J = 8 Hz, 6 H), 1.4 (d, J = 7 Hz, 3 H), 2.6 (br s, 1 H), 3.5 (q, J = 8 Hz, 4 H), 4.8 (q, J = 7 Hz, 1 H), 5.4 (s, 1 H), 7.4 (m, 4 H).

4-(Diethoxymethyl)acetophenone. 106 (223 g, 1.0 mol) and manganese dioxide (480 g, 5.5 mol) were combined in toluene (2.5 L) and the resulting dark suspension was heated to reflux for 18 h, cooled to room temperature, and filtered over diatomaceous earth with ethyl acetate wash. The filtrate was concentrated to yield an oil which was distilled to give the title product (134 g, 60%, bp 113–115 °C at 0.2–0.7 mmHg): ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.2 (t, J = 8 Hz, 6 H), 2.6 (s, 3 H), 3.6 (q, J = 8 Hz, 4 H), 5.6 (s, 1 H), 7.6 (d, J = 9 Hz, 2 H), 8.0 (d, J = 9 Hz, 2 H).

Ethyl 2-[4-(Diethoxymethyl)benzoyl]acetate (86). Sodium hydride (32.4 g, 1.35 mol) was added to ice-cooled ether (400 mL), followed immediately by diethyl carbonate (96 g, 0.81 mol). After stirring for 25 min at room temperature, a solution of 4-(diethoxymethyl)acetophenone (120 g, 0.54 mol) and ethanol (1 mL) in ether (300 mL) was added over 25 min at room temperature. The mixture was slowly heated to reflux and kept at reflux for 6 h. The reaction mixture was cooled to room temperature and then slowly poured into a cold (0 °C) mixture of 10% HCl (500 mL) and ether (500 mL). The aqueous layer was separated and extracted with ether (500 mL), and the organic layers were combined, washed with water (500 mL) and brine (500 mL), dried over magnesium sulfate, and concentrated to yield the product as a viscous oil (158 g, 99%): ^{1}H NMR (60 MHz, $CDCl_3$) δ 1.2 (t, J = 8 Hz, 6 H), 3.6 (q, J = 8 Hz, 4 H), 4.0 (s, 2 H), 4.2 (q, J = 7 Hz, 2 H), 5.6 (s, 1 H), 7.6 (d, J = 8 Hz, 2 H), 8.0 (d, J = 8 Hz, 2 H).

4-[3-(5-Methyl-2-phenyl-4-oxazolyl)propionyl]benzaldehyde (87). Sodium hydride (3.4 g, 0.14 mol) was combined with 250 mL of THF and cooled to 0 °C. With stirring, a solution of 86 (41.5 g, 0.14 mol) in THF (250 mL) was added portionwise over 0.5 h, maintaining the temperature below 25 °C. After stirring for an additional 0.5 h at room temperature, (5-methyl-2-phenyl-4-oxazolyl)methyl chloride (85)[24] (26 g, 0.13 mol) was added, and the mixture was heated to reflux for 48 h, cooled, and concentrated. The residue was taken up in a mixture of acetic acid (360 mL) and concentrated HCl (90 mL), heated to reflux for 5 h, cooled to room temperature, diluted with water (600 mL), and extracted with 1:1 ethyl acetate/ether (2 × 1 L). The organic layers were combined, washed with water (1 L) and brine (1 L), dried over magnesium sulfate, and concentrated. The residue was purified by flash chromatography (ether/chloroform, 1:19) and the product isolated as an oil which solidified on standing (34 g, 85%, mp 76–80 °C): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.4 (s, 3 H), 3.0 (t, J = 6 Hz, 2 H), 3.45 (t, J = 6 Hz, 2 H), 7.4 (m, 3 H), 7.9 (m, 4 H), 8.1 (m, 2 H), 10.1 (s, 1 H).

5-[[4-[3-(5-Methyl-2-phenyl-4-oxazolyl)propionyl]-phenyl]methylene]thiazolidine-2,4-dione (75). 87 (16 g, 50 mmol), thiazolidine-2,4-dione (11.7 g, 0.10 mol), and piperidine (0.85 g, 10 mmol) were combined in ethanol (300 mL), and the mixture was refluxed for 24 h, cooled to 0 °C, and diluted with ether (600 mL). After stirring for 1 h at 0 °C, the precipitate was filtered and triturated with warm (40–50 °C) acetic acid (0.15 L). The resulting slurry was cooled to room temperature and diluted with ether (0.3 L) and the product was collected (14.2 g, 71%, mp 224–225 °C). Anal. ($C_{23}H_{18}N_2O_4S$) C, H, N.

5-[4-[3-(5-Methyl-2-phenyl-4-oxazolyl)propionyl]-benzyl]thiazolidine-2,4-dione (32). 75 (14.2 g) was hydrogenated in THF (800 mL) in the presence of palladium on carbon (10 g) in a Parr shaker at 50 psi and room temperature for 24 h. The catalyst was recovered by filtration over diatomaceous earth with THF wash. The combined filtrate/wash was concentrated to a gum which was crystallized by trituration with 1:1 hexane/ethyl acetate (250 mL) (11.4 g, 81%, mp 145–146 °C): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.35 (s, 3 H), 2.9 (t, J = 7 Hz, 2 H), 3.22 (dd, J = 14, 10 Hz, 1 H), 3.35 (t, J = 7 Hz, 2 H), 3.5 (dd, J = 14, 4 Hz, 1 H), 4.5 (dd, J = 10, 4 Hz, 1 H), 7.3 (d, J = 8 Hz, 2 H), 7.4 (m, 3 H), 7.9 (m, 4 H), 8.32 (br s, 1 H); MS (EI) m/e 420 (M$^+$, 2), 186 (100); UV λ_{max} = 253 nm (MeOH). Anal. ($C_{23}H_{20}N_2O_4S$) C, H, N.

5-[4-[3-(5-Methyl-2-phenyl-4-oxazolyl)-1-hydroxypropyl]benzyl]thiazolidine-2,4-dione (50). 32 (0.70 g) was suspended in 2-propanol (50 mL). Sodium borohydride (0.15 g) was added and the mixture was stirred for 2 h, concentrated to low volume, diluted with water (50 mL), and extracted with ethyl acetate (2 × 200 mL). The combined organic layers were washed with brine, dried over magnesium sulfate, and concentrated, and the residue was chromatographed on silica gel (hexanes/ethyl acetate, 1:1, 1% acetic acid) to yield 50 as a solid (0.32 g, 46%, mp 50–55 °C): ^{1}H NMR (300 MHz, DMSO-d_6) δ 1.92 (m, 2 H), 2.24 (s, 3 H), 2.48 (t, J = 7.8 Hz, 2 H), 3.11 (dd, J = 14, 9.6 Hz, 1 H), 3.39 (dd, J = 14.2, 4.0 Hz, 1 H), 4.56 (m, 1 H), 4.87 (dd, J = 8.8, 3.6 Hz, 1 H), 5.29 (m, 1 H), 7.19 (d, J = 7.2 Hz, 2 H), 7.29 (d, J = 7.3 Hz, 2 H), 7.43–7.48 (m, 3 H), 7.87–7.90 (m, 2 H). Anal. ($C_{23}H_{21}N_2NaO_4S$) C, H, N.

5-[4-[3-(5-Methyl-2-phenyl-4-oxazolyl)-1-propenyl]-benzyl]thiazolidine-2,4-dione (51). A solution of 50 (7.3 g, 17 mmol) and p-toluenesulfonic acid hydrate (1.0 g, 5.3 mmol) in toluene (150 mL) was heated to reflux overnight. The solution was diluted with ethyl acetate (150 mL), washed with water (2 × 150 mL) and brine (150 mL), dried over sodium sulfate, and concentrated. The product was purified by flash chromatography (hexanes/ethyl acetate, 3:2) and obtained as a gummy solid (3.5 g, 50%). The product was dissolved in ethyl acetate (150 mL)

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and sodium 2-ethylhexanoate (1.5 g, 3.7 mmol) was added in ethyl acetate (50 mL). The sodium salt was collected and washed with ether (2.6 g, mp 288 °C): ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.36 (s, 3 H), 2.65 (dd, J = 13.8, 10.5 Hz, 1 H), 3.38 (t, J = 6 Hz, 2 H), 4.13 (dd, J = 10.4, 3.3 Hz, 1 H), 6.2 (dt, J = 16, 6 Hz, 1 H), 6.47 (d, J = 16.2 Hz, 1 H), 7.12 (d, J = 8.3 Hz, 2 H), 7.29 (d, J = 7.9 Hz, 2 H), 7.46–7.50 (m, 3 H), 7.89–7.92 (m, 2 H); ^{13}C NMR (75 MHz, DMSO-d_6) δ 9.95, 28.99, 39.67, 58.02, 125.48, 125.88, 126.72, 126.23, 127.50, 129.07, 129.11, 130.03, 130.51, 134.16, 135.03, 138.95, 144.21, 158.39, 180.58; IR (KBr) ν (cm^{-1}) 1240, 1330, 1550, 1570 (s), 1670; MS (EI) m/e 404 (M$^+$), 288, 173, 105. Anal. ($C_{23}H_{19}N_2NaO_3S\cdot H_2O$) C, H, N.

5-[4-[3-(5-Methyl-2-phenyl-4-oxazolyl)propyl]benzyl]-thiazolidine-2,4-dione (52). A solution of **51** (0.5 g, 1.2 mmol) in ethyl acetate (40 mL) containing 5% palladium on charcoal (0.45 g) was hydrogenated at 40 psi in a Parr apparatus overnight. The catalyst was filtered over diatomaceous earth, the solvent was evaporated, and the residue was purified by flash chromatography (hexanes/ethyl acetate, 3:2). **52** was obtained as a white solid (0.40 g, 80%, mp 98–99 °C): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.97 (quin, J = 7.6 Hz, 2 H), 2.27 (s, 3 H), 2.50 (t, J = 7.5 Hz, 2 H), 2.64 (t, J = 7.7 Hz, 2 H), 3.05 (dd, J = 14.0, 9.9 Hz, 1 H), 3.48 (dd, J = 14.1, 3.8 Hz, 1 H), 4.46 (dd, J = 9.9, 3.9 Hz, 1 H), 7.12 (*AB*, J = 9.0 Hz, 1 H), 7.14 (*AB*, J = 9.0 Hz, 1 H), 7.38–7.44 (m, 3 H), 7.95–7.98 (m, 2 H), 9.73 (br s, 1 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 10.2, 25.2, 30.4, 34.9, 38.3, 53.6, 126.0, 126.6, 127.8, 128.7, 129.0, 129.1, 129.8, 133.3, 135.7, 141.6, 143.5, 159.4, 171.0, 174.8; IR (KBr) ν (cm^{-1}) 720, 1130, 1340, 1700 (s), 1750, 2950; MS (EI) m/e 406 (M$^+$), 174, 173, 172, 145, 117, 105, 104, 77, 70. Anal. ($C_{23}H_{22}N_2O_3S$) C, H, N.

5-[4-[3-(5-Methyl-2-phenyl-4-oxazolyl)-1-(methoxyimino)propyl]benzyl]thiazolidine-2,4-dione (73). **32** (0.10 g, 0.24 mmol), methoxyamine hydrochloride (50 mg, 0.60 mmol), and pyridine (2 mL) were combined in ethanol (3 mL) and the mixture was stirred at room temperature for 18 h and then concentrated. The residue was taken up in ethyl acetate (7.5 mL), washed with cold 18% HCl (5 mL), and brine (5 mL) and concentrated to yield **73** as a white solid which was recrystallized from ethyl acetate/hexanes (mp 138–140 °C): ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.18 (s, 3 H), 2.64 (t, J = 7 Hz, 2 H), 2.99 (t, J = 7 Hz, 2 H), 3.10 (dd, J = 14, 9 Hz, 1 H), 3.35 (dd, J = 14, 4 Hz, 1 H), 3.88 (s, 3 H), 4.88 (dd, J = 9, 4 Hz, 1 H), 7.22 (d, J = 8 Hz, 2 H), 7.46–7.48 (m, 3 H), 7.55 (d, J = 8 Hz, 2 H), 7.86–7.90 (m, 2 H). Anal. ($C_{24}H_{23}N_3O_4S\cdot{}^1/_2H_2O$) C, H, N.

5-Benzylthiazolidine-2,4-dione (88). A solution of 5-(phenylmethylene)thiazolidine-2,4-dione (25 g, 0.12 mol) in THF (750 mL) and acetic acid (250 mL) was hydrogenated in a Parr shaker over 10% Pd–C (25 g of 50% wt water). The catalyst was removed by filtration over diatomaceous earth and the solvent evaporated. The solid was recrystallized from ethanol/water (1:2) and obtained as pale grey crystals (15 g, 60%, mp 101–103 °C).

4-[(2,4-Dioxothiazolidin-5-yl)methyl]benzenesulfonyl Chloride (89). Chlorosulfonic acid (5 mL) was cooled to 0 °C and **88** (9.6 mmol, 2.0 g) was added portionwise. The reaction mixture was stirred at room temperature for 0.5 h and poured into ice (25 g). The solution was extracted with methylene chloride (2 × 50 mL), the combined organic layers were dried over sodium sulfate, and the solvent was removed to afford the product which was used without further purification.

Ethyl 5-Methyl-3-oxo-2-phenyloxazole-4-carboxylate Hydrochloride (107). To a solution of ethyl 2-(hydroxyimino)-3-oxobutyrate[25] (340 g, 2.2 mol) in acetic acid (550 mL) was added benzaldehyde (290 mL, 2.8 mol). The mixture was cooled to 0 °C and dry HCl was bubbled into the stirred reaction mixture at a moderate rate for 2 h. The mixture was diluted with 3 volumes of ether and filtered to yield 620 g of wet ether product which was immediately bottled and stored at refrigerator temperature.

Ethyl 5-Methyl-2-phenyloxazole-4-carboxylate (90). **107** (210 g) was dissolved in ethanol (1 L) and methanol (120 mL) and hydrogenated in a Parr shaker over 10% Pd–C (14 g) at 50 psi for 3 h, by which time uptake of hydrogen was complete. The catalyst was filtered over diatomaceous earth with methanol wash, and the solvent was removed to yield the product as an oil (67 g, 40%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.42 (t, J = 7 Hz, 3 H), 2.70 (s, 3 H), 4.42 (q, J = 7 Hz, 2 H), 7.42–7.45 (m, 3 H), 8.05–8.08 (m, 2 H).

5-Methyl-2-phenyloxazole-4-methanol (108). A slurry of $LiAlH_4$ (11 g, 0.29 mol) in ether (300 mL) was cooled to 0 °C and a solution of **90** (67 g, 0.29 mol) in ether (300 mL) was added over 30 min, maintaining the temperature at 0–10 °C. The reaction was stirred for 1 h at room temperature and then diluted with THF (200 mL) and carefully quenched with water (11 mL), 1 N NaOH (11 mL), and again water (33 mL). The mixture was stirred for 15 min, diluted with THF (200 mL), and filtered over diatomaceous earth and the filtrate was concentrated to yield the title product as a solid (46 g, 84%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.43 (s, 3 H), 3.46 (br, 1 H), 4.64 (s, 2 H), 7.43–7.46 (m, 3 H), 8.00–8.03 (m, 2 H).

5-Methyl-2-phenyloxazole-4-carboxaldehyde (109). To a solution of **108** (20 g, 0.11 mol) in dichloromethane (500 mL) was added pyridinium dichromate (120 g, 0.32 mol). The slurry was stirred for 7 h, diluted with ether (1 L), and filtered over diatomaceous earth and the filtrate was concentrated to yield the product as a solid (14 g, 70%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.72 (s, 3 H), 7.45–7.48 (m, 3 H), 8.02–8.06 (m, 2 H), 10.0 (s, 1 H).

***N*-Methyl-*N*-[(5-methyl-2-phenyl-4-oxazolyl)methyl]-amine (91).** **109** (2.0 g, 11 mmol) was dissolved in ether (50 mL), magnesium sulfate (2.0 g) was added, and the mixture was cooled to 0 °C and saturated with gaseous methylamine. The mixture was stirred for 15 min at 0°C and then at room temperature for 3 h and filtered and the filtrate was concentrated. The residue was taken up in methanol (50 mL) and cooled to 0 °C and sodium borohydride (2.2 g, 58 mmol) was added. The mixture was stirred at 0 °C for 15 min and then at room temperature for 18 h. It was then diluted with 2 volumes of water and extracted with ethyl acetate (2 × 150 mL). The combined extracts were washed with water (2 × 150 mL) and brine (150 mL) and concentrated to yield the product as an oil (1.5 g, 46%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.38 (s, 3 H), 2.45 (s, 3 H), 7.38–7.41 (m, 3 H), 7.94–7.98 (m, 2 H).

4-[*N*-Methyl-*N*-[(5-methyl-2-phenyl-4-oxazolyl)]methylaminocarbonyl]benzaldehyde (110). To an ice-cooled solution of 4-carboxybenzaldehyde (1.1 g, 7.4 mmol) and triethylamine (1.0 mL, 7.4 mmol) in THF (50 mL) were added isobutyl chloroformate (0.96 mL, 7.4 mmol) and after 30 min a solution of **91** (1.5 g, 7.4 mmol) in THF (30 mL). The mixture was stirred for 30 min at 0 °C and then at room temperature overnight. Water was added followed by 1 N NaOH and the mixture was extracted with ethyl acetate (2×). The combined extracts were washed with water and brine, dried over magnesium sulfate, and concentrated to an oil. The product was purified by flash chromatography (hexanes/ethyl acetate, 1:3) and obtained as an oil (0.52 g, 21%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.20 (s, 1.5 H), 2.48 (s, 1.5 H), 3.05 (s, 3 H), 4.24 (s, 1 H), 4.62 (s, 2 H), 7.37–7.42 (m, 3 H), 7.56 (d, J = 8 Hz, 1 H), 7.80–7.98 (m, 5 H), 9.98 (s, 1 H).

***N*-Methyl-*N*-[(5-methyl-2-phenyl-4-oxazolyl)methyl]-4-[(2,4-dioxothiazolidin-5-ylidene)methyl]benzamide (111).** A mixture of **110** (0.52 g, 1.6 mmol), 2,4-thiazolidinedione (0.27 g, 2.3 mmol), and sodium acetate (0.38 g, 4.7 mmol) was heated to 140 °C for 2 h and then triturated in water, and the resulting solid was collected, washed with water, and dried (0.61 g, 90%, mp 95–98 °C): ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.50 (s, 3 H), 2.95 (s, 3 H), 4.32 (br s, 1 H), 4.58 (br s, 1 H), 7.48–7.55 (m, 5 H), 7.62–7.75 (m, 3 H), 7.93–7.96 (m, 2 H); MS (EI) m/e 433 (M$^+$), 201 (100), 193, 172, 117, 105, 104.

***N*-Methyl-*N*-[(5-methyl-2-phenyl-4-oxazolyl)methyl]-4-[(2,4-dioxothiazolidin-5-yl)methyl]benzamide (64).** A solution of **111** (0.30 g, 0.69 mmol) in THF (80 mL) and acetic acid (25 mL) was hydrogenated over 10% Pd–C sulfur-resistant catalyst (0.80 g) in a Parr shaker at 50 psi for 2 h. The catalyst was filtered, the solvent was evaporated, and the product was purified by flash chromatography (hexanes/ethyl acetate, 1:3) and obtained as a solid (81 mg, 27%, mp 75–78 °C); IR (KBr) ν (cm^{-1}) 1610, 1700 (s), 1750, 3200 (br); MS (EI) m/e 435 (M$^+$), 313, 202, 201, 172. Anal. ($C_{23}H_{21}N_3O_4S\cdot H_2O$) C, H, N.

***N*-Methyl-*N*-[(5-methyl-2-phenyl-4-oxazolyl)methyl]-4-[(2,4-dioxothiazolidin-5-yl)methyl]benzenesulfonamide (70).**

(25) Adkins, H.; Reeve, E. W. A Synthesis of *dl*-Threonine. *J. Am. Chem. Soc.* 1938, *60*, 1328–1331.

To an ice-cooled solution of **91** (0.59 g, 1.9 mmol) in dichloromethane (20 mL) was added a solution of **89** (0.39 g, 1.9 mmol) in dichloromethane (10 mL) followed by diisopropylethylamine (0.40 mL, 2.3 mmol). The solution was stirred at 0 °C for 15 min and then at room temperature overnight. The solution was diluted with dichloromethane, washed with 1 N HCl, 5% sodium bicarbonate (2×), and brine, dried over magnesium sulfate, and concentrated to a yellow solid which was purified by flash chromatography (chloroform/methanol, 20:1) and obtained as a white solid (0.45 g, 50%, mp 83–84 °C): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.41 (s, 3 H), 2.81 (s, 3 H), 3.13 (dd, J = 14.1, 9.4 Hz, 1 H), 3.46 (dd, J = 14.1, 3.9 Hz, 1 H), 4.43 (dd, J = 9.4, 4.0 Hz, 1 H), 7.34 (d, J = 8.3 Hz, 2 H), 7.39–7.42 (m, 3 H), 7.58 (d, J = 8.3 Hz, 2 H), 7.87–7.90 (m, 2 H), 9.49 (br s); IR (KBr) ν (cm^{-1}) 1160, 1340, 1705, 1760, 3210 (br); MS (EI) m/e 471 (M^+), 201, 172. Anal. ($C_{22}H_{21}N_3O_5S_2 \cdot {}^1/_4H_2O$) C, H, N.

Methyl 3-Benzamido-4-oxovalerate (93). L-Aspartic acid β-methyl ester hydrochloride (**92**) (5.0 g, 27 mmol) was partially dissolved in pyridine (15 mL) and the mixture cooled to 0 °C. Benzoyl chloride (3.1 mL, 27 mmol) was then added dropwise and stirring was continued for 1.5 h at 0 °C and 0.5 h at room temperature. Acetic anhydride (10 mL) was added and the mixture was heated to 90 °C for 2 h and then diluted with water (15 mL) and heating was continued for 15 min. The mixture was cooled, acidified with excess dilute HCl, and extracted with ethyl acetate (2 × 75 mL). The combined organic layers were washed with 2 N HCl (50 mL), water (50 mL), saturated sodium bicarbonate (3 × 50 mL), water (50 mL), and brine (50 mL), dried over sodium sulfate, and concentrated to yield a thick oil (4.3 g) which was used directly in the next step.

Methyl 2-(5-Methyl-2-phenyl-4-oxazolyl)acetate (112). Phosphorus oxychloride (20 mL) was added to a solution of the crude **93** (4.3 g) in toluene (80 mL) and the mixture was heated to reflux for 4 h, cooled to room temperature, and poured into ice/water (200 mL). The resulting mixture was adjusted to pH 7.5 with potassium carbonate and extracted with ether (2 × 100 mL). The combined organic layers were combined, washed with water (100 mL) and brine (100 mL), and concentrated. The product was isolated by flash chromatography (hexanes/ethyl acetate, 2:1) as an oil (1.1 g, 18%): ^{1}H NMR (60 MHz, $CDCl_3$) δ 2.3 (s, 3 H), 3.5 (s, 2 H), 3.7 (s, 3 H), 7.1–7.4 (m, 3 H), 7.7–7.9 (m, 2 H).

2-(5-Methyl-2-phenyl-4-oxazolyl)acetic Acid (94). **112** (1.1 g, 4.8 mmol) was slurried in 1 N NaOH (15 mL) and heated to gentle reflux for 0.5 h. The resulting solution was cooled to 0–5 °C and acidified with 6 N HCl. The precipitate was collected and dried (0.82 g, 80%, mp 120.5–124.5 °C): ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.32 (s, 3 H), 3.50 (s, 2 H), 7.40–7.45 (m, 3 H), 7.85–7.90 (m, 2 H), 12.4 (br s, 1 H).

5-[4-[2-(5-Methyl-2-phenyl-4-oxazolyl)acetamido]benzyl]thiazolidine-2,4-dione (66). **94** (0.40 g, 1.8 mmol) was dissolved in dichloromethane (5 mL) and cooled to 0–5 °C. Triethylamine (0.22 mL) was added dropwise followed by ethyl chloroformate (0.31 mL). After 15 min of stirring at 0 °C, a solution of 5-(4-aminobenzyl)thiazolidine-2,4-dione[12] (0.71 g, 3.2 mmol) and triethylamine (0.24 mL) in dichloromethane (15 mL) was added. The cold bath was removed and the solution was stirred at room temperature overnight. The solvent was removed and the residue was partitioned between 2 N HCl (25 mL) and ethyl acetate (25 mL). The aqueous layer was extracted with additional ethyl acetate (25 mL) and the combined organic layers were washed with water (30 mL), saturated sodium bicarbonate (30 mL), and brine (30 mL), dried over sodium sulfate, and concentrated. The product was isolated by flash chromatography (hexanes/ethyl acetate, 2:1) as a solid (0.43 g, 55%, mp 201–202 °C): ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.40 (s, 3 H), 3.07 (dd, J = 14.2, 9.2 Hz, 1 H), 3.33 (dd, 1 H), 3.60 (s, 2 H), 4.95 (dd, J = 9.1, 4.3 Hz, 1 H), 7.18 (d, J = 8.5 Hz, 2 H), 7.49–7.51 (m, 3 H), 7.56 (d, J = 8.5 Hz, 2 H), 7.89–7.93 (m, 2 H), 10.20 (s, 1 H), 12.0 (br s, 1 H); IR (KBr) ν (cm^{-1}) 720, 1520, 1545, 1660, 1680 (s), 1755; MS (EI) m/e 421 (M^+), 174, 173, 172, 106, 104, 70. Anal. ($C_{22}H_{19}N_3O_4S \cdot {}^1/_2H_2O$) C, H, N.

1-[4-(Diethoxymethyl)phenyl]-2-phenylethanol (113). A solution of terephthalaldehyde mono(diethyl acetal) (**95**) (16 g, 75 mmol) in ether (200 mL) was cooled to −30 °C and benzylmagnesium bromide (56 mL of a 2 M solution, 0.11 mol) was added dropwise, keeping the temperature below −20 °C. After 1 h at −20 °C the mixture was warmed to 0 °C, poured into saturated ammonium chloride (300 mL), and extracted with ether (2 × 300 mL). The combined extracts were washed with water and brine, dried over magnesium sulfate, and concentrated. The crude product was plug-filtered through silica gel using hexane/ether (2:1) as a solvent. The product was obtained as an oil (15.7 g, 69%). Crystallization in cold hexane gave the pure compound as a white solid, mp 48–50 °C.

4-(1-Hydroxy-2-phenylethyl)benzaldehyde (96). 113 (8.9 g, 30 mmol) was dissolved in THF (300 mL) and 3.5% perchloric acid (180 mL) and the solution was stirred at room temperature overnight. The mixture was concentrated to ca. 150 mL and extracted with ethyl acetate (2 × 300 mL). The combined organic layers were washed with water (250 mL), saturated sodium bicarbonate, and brine, dried over magnesium sulfate and concentrated to a colorless syrup (6.9 g, 100%): ^{1}H NMR (60 MHz, $CDCl_3$) δ 2.6 (s, 1 H), 2.9 (d, J = 7 Hz, 2 H), 4.9 (t, J = 7 Hz, 1 H), 7.1–7.2 (m, 5 H), 7.3 (d, J = 8 Hz, 2 H), 7.7 (d, J = 8 Hz, 2 H), 10.0 (s, 1 H).

5-[[4-(1-Hydroxy-2-phenylethyl)phenyl]methylene]thiazolidine-2,4-dione (114) was prepared by condensation with 2,4-thiazolidinedione as described above for **111**. Yield: 7.5 g (75%). Mp 203–205 °C.

5-[4-(1-Hydroxy-2-phenylethyl)benzyl]thiazolidine-2,4-dione (115). To a suspension of **114** (7.0 g, 21 mmol) in methanol (750 mL) was added 3% sodium amalgam (42 g), and the mixture was stirred overnight and then filtered over diatomaceous earth and concentrated. The residue was purified by plug filtration through silica gel (600 g) using hexanes/ethyl acetate (1:1) with 2.5% AcOH as solvent. The product was dissolved in ethyl acetate, washed with saturated sodium bicarbonate, water, and brine, dried over magnesium sulfate, and concentrated to a light yellow gum (4.0 g, 57%).

5-[4-(1-Oxo-2-phenylethyl)benzyl]thiazolidine-2,4-dione (4). An aqueous chromic acid solution was prepared by dissolving sodium dichromate (10 g) in water (30 mL), adding concentrated sulfuric acid (13.6 g) at 0 °C, and diluting with water to a total volume of 50 mL. **115** (1.1 g, 3.3 mmol) was dissolved in ether (30 mL) and the oxidizing reagent (4 mL) was added dropwise at 0 °C. The mixture was warmed to room temperature and after 2 h was diluted with ether (100 mL) and water (25 mL). The aqueous layer was extracted with ether (25 mL) and the combined organic phases were washed with water and brine, dried over magnesium sulfate, and concentrated to a gum. The product was purified by column chromatography on silica gel (100 g) using hexanes/ethyl acetate (2:1) with 2.5% acetic acid as eluent and crystallized from hexanes/ethyl acetate to give a white solid (0.27 g, 25%, mp 145–146 °C): ^{1}H NMR (sodium salt, 300 MHz, DMSO-d_6) δ 3.07 (dd, J = 14, 9 Hz, 1 H), 3.46 (dd, J = 14, 4 Hz, 1 H), 4.23 (dd, J = 9, 4 Hz, 1 H), 4.38 (s, 2 H), 7.24–7.32 (m, 5 H), 7.37 (d, J = 8 Hz, 1 H), 7.96 (d, J = 8 Hz, 1 H). Anal. ($C_{18}H_{15}NO_3S$) C, H, N.

1-(4-Bromophenyl)-2-phenoxyethanol (98). A mixture of phenol (1.4 g, 15 mmol), 4′-bromophenacyl bromide (**97**) (4.2 g, 15 mmol), and potassium carbonate (4.2 g, 30 mmol) in acetone (50 mL) was heated to reflux for 8 h. The solution was concentrated, diluted with water (50 mL), and extracted with ether (2 × 100 mL). The combined extracts were washed with 10% NaOH and brine, dried over magnesium sulfate, and concentrated. The crude product was dissolved in 2-propanol (25 mL) and dichloromethane (25 mL) and sodium borohydride (0.57 g, 15 mmol) was added at 0 °C. After 1 h water was added and the mixture was extracted with dichloromethane (2×). The combined extracts were washed with brine, dried over magnesium sulfate, and concentrated. The product was plug-filtered through silica gel with dichloromethane as solvent to give an oily solid (3.0 g, 68%) which was used directly in the next step.

1-Bromo-4-[1-[(*tert*-butyldimethylsilyl)oxy]-2-phenoxyethyl]benzene (116). A solution of **98** (2.9 g, 10 mmol), *tert*-butyldimethylsilyl chloride (1.9 g, 12.5 mmol), and imidazole (1.7 g, 25 mmol) in DMF (40 mL) was stirred at room temperature for 36 h. Sodium bicarbonate (10%) (150 mL) was added and the mixture was extracted with hexanes (2 × 200 mL). The combined extracts were washed with water and brine, dried over magnesium sulfate, and concentrated. The residue was purified

by plug-filtration through silica gel using 20% butyl chloride in hexanes as the solvent. The title product was obtained as a colorless oil (2.5 g, 61%): ^{1}H NMR (60 MHz, $CDCl_3$) δ 0.4 (s, 3 H), 0.6 (s, 3 H), 1.45 (s, 9 H), 3.9 (d, J = 7 Hz, 2 H), 5.3 (t, J = 7 Hz, 1 H), 7.7 (AB, J = 9 Hz, 2 H), 7.9 (AB, J = 9 Hz, 2 H).

4-[1-[(*tert*-Butyldimethylsilyl)oxy]-2-phenoxyethyl]benzaldehyde (99). A solution of 116 (2.5 g, 6.1 mmol) in THF (25 mL) was cooled to −78 °C and treated with *n*-BuLi (3.35 mL of a 2 M solution in hexanes, 6.7 mmol). The solution was stirred at −78 °C for 1 h and then DMF (0.52 mL, 6.7 mmol) was added and the solution was stirred for another hour at −78 °C, quenched with 10% HCl, warmed to room temperature, and extracted with ethyl acetate (2×). The combined extracts were washed with brine, dried over magnesium sulfate, and concentrated. The product (2.0 g) was used in the next step without further purification.

5-[4-[1-[(*tert*-Butyldimethylsilyl)oxy]-2-phenoxyethyl]benzyl]thiazolidine-2,4-dione (117). 5-[4-[1-[(*tert*-Butyldimethylsilyl)oxy]-2-phenoxyethyl]phenylmethylene]thiazolidine-2,4-dione (118) was prepared by condensation with 2,4-thiazolidinedione as described above for 111. The crude product was dissolved in methanol (100 mL) and 3% sodium amalgam (20 g) was added. The mixture was stirred for 20 h, filtered through diatomaceous earth, and concentrated. The residue was taken up in ethyl acetate (500 mL), washed with cold 10% HCl and brine, dried over magnesium sulfate, and concentrated. The product was purified by plug filtration through silica gel using hexanes/ethyl acetate (2:1) as solvent and obtained as an oil (1.6 g, 55% for 3 steps): ^{1}H NMR (60 MHz, $CDCl_3$) δ 0.2 (s, 3 H), 0.6 (s, 3 H), 0.95 (s, 9 H), 3.05 (dd, J = 14, 9 Hz, 1 H), 3.5 (dd, J = 9, 4 Hz, 1 H), 3.95 (d, J = 6 Hz, 2 H), 4.5 (dd, J = 9, 4 Hz, 1 H), 5.0 (t, J = 6 Hz, 1 H), 6.8–7.5 (m, 9 H).

5-[4-(1-Hydroxy-2-phenoxyethyl)benzyl]thiazolidine-2,4-dione (119). A solution of 117 (1.5 g, 3.1 mmol) in THF (50 mL) and 3.5% perchloric acid (30 mL) was stirred at room temperature overnight. It was then extracted with ethyl acetate (2×), and the combined extracts were washed with brine, dried over magnesium sulfate, and concentrated. The product was purified by plug filtration through silica gel using hexanes/ethyl acetate (2:1) as solvent and obtained as an oil (0.69 g, 64%).

5-[4-(1-Oxo-2-phenoxyethyl)benzyl]thiazolidine-2,4-dione (6) was prepared by chromic acid oxidation of 119 as described above for 4 and obtained as a solid (mp 170–174 °C): ^{1}H NMR (300 MHz, DMSO-d_6) δ 3.35 (dd, J = 14, 9 Hz, 1 H), 3.54 (dd, J = 14, 4 Hz, 1 H), 5.07 (dd, J = 9, 4 Hz, 1 H), 5.60 (s, 2 H), 7.00 (m, 3 H), 7.32 (t, J = 8 Hz, 2 H), 7.50 (d, J = 8 Hz, 2 H), 8.03 (d, J = 8 Hz, 2 H). Anal. ($C_{18}H_{15}NO_4S$) C, H, N.

2-(5-Methyl-2-phenyl-4-oxazolyl)ethanol (120) was prepared by lithium aluminum hydride reduction of methyl 2-(5-methyl-2-phenyl-4-oxazolyl)acetate (100) (1.1 g) as described above for 108 and obtained as a solid (0.75 g, 78%, mp 58–60 °C): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.36 (s, 3 H), 2.75 (t, J = 6 Hz, 2 H), 3.95 (t, J = 6 Hz, 2 H), 7.42–7.45 (m, 3 H), 7.98–8.01 (m, 2 H).

4-(2-Bromoethyl)-5-methyl-2-phenyloxazole (121). To an ice-cooled solution of 120 (0.75 g, 3.7 mmol) and carbon tetrabromide (2.5 g, 7.4 mmol) in ether (30 mL) was added triphenylphosphine (1.9 g, 7.4 mmol). The mixture was stirred for 10 min at 0 °C and 3 h at room temperature and then filtered and concentrated. The residue was purified by plug filtration (silica gel, dichloromethane) and the product obtained as an oil which solidified on standing (0.34 g, 35%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.36 (s, 3 H), 3.05 (t, J = 7 Hz, 2 H), 3.66 (t, J = 7 Hz, 2 H), 7.39–7.42 (m, 3 H), 7.94–7.97 (m, 2 H).

4-[2-[(4-Bromophenyl)thio]ethyl]-5-methyl-2-phenyloxazole (101). To an ice-cooled suspension of sodium hydride (53 mg, 2.2 mmol) in THF (10 mL) were added 4-bromothiophenol (0.32 g, 1.7 mmol) and after 15 min a solution of 121 (0.34 g, 1.3 mmol) in THF (5 mL). The mixture was stirred at 0 °C for 1 h and at room temperature for 2 h. The precipitate was filtered, and the filtrate was diluted with ethyl acetate, washed with brine, dried over magnesium sulfate, and concentrated. The product was purified by plug filtration (silica gel, hexanes/ether, 4:1) and obtained as a solid (0.42 g, 86%, mp 48–50 °C): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.37 (s, 3 H), 2.80 (t, J = 7 Hz, 2 H), 3.25 (t, J = 7 Hz, 2 H), 7.18 (d, J = 8 Hz, 2 H), 7.35 (d, J = 8 Hz, 2 H), 7.39–7.42 (m, 3 H), 7.92–7.96 (m, 2 H).

4-[[2-(5-Methyl-2-phenyl-4-oxazolyl)ethyl]thio]benzaldehyde (122) was prepared by transmetalation and formylation of 101 as described for 99 and obtained as a white solid (0.25 g, 70%, mp 77–79 °C): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.35 (s, 3 H), 2.86 (t, J = 7 Hz, 2 H), 3.33 (t, J = 7 Hz, 2 H), 7.34 (d, J = 8 Hz, 2 H), 7.37–7.40 (m, 3 H), 7.69 (d, J = 8 Hz, 2 H), 7.90–7.94 (m, 2 H), 9.82 (s, 1 H).

5-[[4-[[2-(5-Methyl-2-phenyl-4-oxazolyl)ethyl]thio]phenyl]methylene]thiazolidine-2,4-dione (123). A solution of 122 (0.25 g, 0.77 mmol), 2,4-thiazolidinedione (0.18 g, 1.55 mmol), and piperidine (14 mg, 0.16 mmol) in ethanol (10 mL) was heated to reflux for 24 h. The mixture was cooled, ether (10 mL) was added, and the precipitate was collected (0.16 g, 49%, mp 193–195 °C): ^{1}H NMR (300 MHz, DMSO-d_6) δ 2.37 (s, 3 H), 2.83 (t, J = 7 Hz, 2 H), 3.35 (t, J = 7 Hz, 2 H), 7.41–7.50 (m, 3 H), 7.69 (s, 1 H), 7.86–7.90 (m, 2 H).

5-[4-[[2-(5-Methyl-2-phenyl-4-oxazolyl)ethyl]thio]benzyl]thiazolidine-2,4-dione (60) was prepared by sodium amalgam reduction of 123 as described for 117 and obtained as a solid (86 mg, 57%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.35 (s, 3 H), 2.83 (t, J = 7 Hz, 2 H), 3.10 (dd, J = 14, 9 Hz, 1 H), 3.30 (t, J = 7 Hz, 2 H), 3.43 (dd, J = 9, 4 Hz, 1 H), 4.46 (dd, J = 9, 4 Hz, 1 H), 7.12 (d, J = 8 Hz, 2 H), 7.29 (d, J = 8 Hz, 2 H), 7.42–7.44 (m, 3 H), 7.94–7.97 (m, 2 H). The compound was converted to its sodium salt as described for 51. Mp 268–270 °C. Anal. ($C_{22}H_{19}N_2NaO_3S_2$) C, H, N.

5-[4-[[2-(5-Methyl-2-phenyl-4-oxazolyl)ethyl]sulfonyl]benzyl]thiazolidine-2,4-dione (61). To an ice-cooled solution of 60 (53 mg, 0.13 mmol) in dichloromethane (5 mL) was added *m*-CPBA (58 mg, 0.28 mmol) in small portions. The mixture was allowed to warm to room temperature and stirred for 2 h, then diluted with dichloromethane, washed with 5% sodium bicarbonate and brine, dried over magnesium sulfate, and concentrated, to yield the product as a white solid (27 mg, 47%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.34 (s, 3 H), 2.95 (q, J = 6 Hz, 2 H), 3.10 (dd, J = 14, 9 Hz, 1 H), 3.36 (dd, J = 9, 4 Hz, 1 H), 3.56 (t, J = 6 Hz, 2 H), 4.40 (dd, J = 9, 4 Hz, 1 H), 7.34 (d, J = 8 Hz, 2 H), 7.40–7.44 (m, 3 H), 7.82 (d, J = 8 Hz, 2 H), 7.84–7.88 (m, 2 H); HRMS calcd 456.0814, found 456.0797.

Methyl 3-(2-Bromo-5-thienyl)-3-oxopropionate (103). To a suspension of sodium hydride (0.17 kg of a 60% dispersion, 4.2 mol) in THF (1 L) were added dimethyl carbonate (0.76 kg, 8.4 mol) and a solution of 2-acetyl-5-bromothiophene[26] (430 g, 2.1 mol) in THF (500 mL), the latter dropwise. The solution was stirred for 1 h and then poured into water, acidified to pH 2 with 6 N HCl, and extracted with ether (3×). The combined extracts were dried over magnesium sulfate and concentrated. The residue was distilled (bp 140–150 °C at 2 mmHg), and the oily distillate was washed with hexanes and dried to give the pure title compound (390 g, 71%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.73 (s, 3 H), 3.86 (s, 2 H), 7.09 (d, J = 4 Hz, 1 H), 7.45 (d, J = 4 Hz, 1 H).

5-Bromo-2-[3-(2-(4-methylphenyl)-5-methyl-4-oxazolyl)propionyl]thiophene (104) was prepared by condensation of 4-(chloromethyl)-5-methyl-2-(4-methylphenyl)oxazole (102) and 103 followed by decarboxylation, as described for 87. The product was obtained as a brown solid (mp 119 °C): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.30 (s, 3 H), 2.34 (s, 3 H), 2.85 (t, J = 7.1 Hz, 2 H), 3.21 (t, J = 7.0 Hz, 2 H), 7.01 (d, J = 3.9 Hz, 1 H), 7.18 (d, J = 7.9 Hz, 2 H), 7.42 (d, J = 3.9 Hz, 1 H), 7.80 (d, J = 8.2 Hz, 2 H). Anal. C, H, N.

5-Bromo-2-[1-hydroxy-3-[2-(4-methylphenyl)-5-methyl-4-oxazolyl]propyl]thiophene (124). To a solution of 104 (120 g, 0.31 mol) in ethanol (1.9 L) was added sodium borohydride (8.0 g, 0.21 mol) by portions. After 1 h the solvent was removed, water was added, and the mixture was acidified to pH 1.5 with 6 N HCl and extracted with chloroform (3×). The combined extracts were washed, dried over magnesium sulfate, and concentrated, leaving a yellow gum which was used directly in the next step: ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.15 (m, 2 H), 2.30 (s, 3 H), 2.38 (s, 3 H), 2.68 (m, 2 H), 4.99 (dd, J = 8.0, 4.3 Hz, 2 H), 6.70 (d, J = 3.8 Hz, 1 H), 6.87 (d, J = 3.7 Hz, 1 H), 7.23 (d, J = 8.1 Hz, 2 H), 7.86 (d,

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J = 8.2 Hz, 2 H); MS (EI) m/e 392 (M^+), 201, 200, 188, 187 (100), 186, 119, 118, 91, 84, 70; IR ($CHCl_3$) ν (cm^{-1}) 1282, 1443, 1500, 1640, 2920.

5-[[5-[1-Hydroxy-3-[2-(4-methylphenyl)-5-methyl-4-oxazolyl]propyl]-2-thienyl]methyl]thiazolidine-2,4-dione (53) was prepared in 5 steps from **124** by the same sequence as that described for **119** and obtained as a white foam: ^1H NMR (300 MHz, $CDCl_3$) δ 2.13 (m, 2 H), 2.31 (s, 3 H), 2.38 (s, 3 H), 2.65 (m, 2 H), 3.39 (dd, J = 51.2, 8.8 Hz, 1 H), 3.59 (dd, J = 15.2, 3.8 Hz, 1 H), 4.50 (dd, J = 8.9, 3.7 Hz, 1 H), 5.01 (m, 1 H), 6.80 (m, 2 H), 7.24 (d, J = 7 Hz, 2 H), 7.88 (d, J = 6.6 Hz, 2 H); MS (EI) m/e 245, 201, 188, 187, 186, 141, 118; IR ($CHCl_3$) ν (cm^{-1}) 1700, 1750; HRMS calcd 428.0858, found 428.0749.

5-[[5-[3-[2-(4-Methylphenyl)-5-methyl-4-oxazolyl]propionyl]-2-thienyl]methyl]thiazolidine-2,4-dione (57) was prepared by PDC oxidation of the alcohol **53**, as described above for **109** and obtained as a solid: mp 158-160 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 2.31 (s, 3 H), 2.36 (s, 3 H), 2.87 (t, J = 6.9 Hz, 2 H), 3.24 (t, J = 6.8 Hz, 2 H), 3.45 (dd, J = 15.1, 8.1 Hz, 1 H), 3.66 (dd, J = 14.9, 4 Hz, 1 H), 4.53 (dd, J = 8, 4 Hz, 1 H), 6.90 (d, J = 3.4 Hz, 1 H), 7.20 (d, J = 7.8 Hz, 2 H), 7.56 (d, J = 3.5 Hz, 1 H), 7.81 (d, J = 8.0 Hz, 2 H), 8.78 (br s, 1 H); MS (EI) m/e 440 (M^+), 200; IR (KBr) ν (cm^{-1}) 1670, 1700, 1750. Anal. ($C_{22}H_{20}N_2O_4S_2^{-1}/_2H_2O$) C, H, N.

5-[[5-[3-[2-(4-Methylphenyl)-5-methyl-4-oxazolyl]-1-propenyl]-2-thienyl]methyl]thiazolidine-2,4-dione (59). A solution of **105** (0.15 kg, 0.27 mol) in THF (600 mL) and 6 N HCl (600 mL) was stirred at room temperature for 45 min. The pH was adjusted to 5 with sodium bicarbonate and the solution was extracted with ethyl acetate (3×). The combined extracts were dried over magnesium sulfate and concentrated. The product was isolated by flash chromatography (30% ethyl acetate in hexanes) as an oil (42 g, 36%): ^1H NMR (300 MHz, $CDCl_3$) δ 2.31 (s, 3 H), 2.36 (s, 3 H), 3.28 (dd, J = 14.5, 8.8 Hz, 1 H), 3.36 (d, J = 6.7 Hz, 2 H), 3.55 (dd, J = 15.0, 3.8 Hz, 1 H), 4.45 (dd, J = 9.0, 3.8 Hz, 1 H), 6.11 (dt, J = 15.4, 7 Hz, 1 H), 6.47 (d, J = 15.4 Hz, 1 H), 6.69 (AB, J = 3.6 Hz, 1 H), 6.70 (AB, J = 3.7 Hz, 1 H), 7.20 (d, J = 7.9 Hz, 2 H), 7.84 (d, J = 8.2 Hz, 2 H), 9.0 (br s, 1 H); MS (EI) m/e 424 (M^+); IR ($CHCl_3$) ν (cm^{-1}) 1700. The product was converted to its sodium salt by the method described for **51**. Anal. ($C_{22}H_{19}N_2NaO_3S_2$) C, H, N.

5-[4-[3-[5-Methyl-2-(4-hydroxy-3,5-dimethylphenyl)-4-oxazolyl]propionyl]benzyl]thiazolidine-2,4-dione (45). A solution of **44** (105 mg, 0.21 mmol) in acetic acid (10 mL) and 48% HBr (5 mL) was heated to reflux for 1 h, cooled, poured into ice-water, and extracted with ethyl acetate. The combined extracts were washed with water, saturated sodium bicarbonate, water again, and brine, dried over magnesium sulfate, and concentrated (103 mg, 100%): ^1H NMR (300 MHz, $CDCl_3$) δ 2.22 (s, 6 H), 2.32 (s, 3 H), 2.86 (t, J = 7 Hz, 2 H), 3.15 (dd, J = 14, 9 Hz, 1 H), 3.30 (t, J = 7 Hz, 2 H), 3.47 (dd, J = 14, 4, Hz, 1 H), 4.49 (dd, J = 8, 4 Hz, 1 H), 7.23 (d, J = 8 Hz, 2 H), 7.56 (s, 2 H), 7.86 (d, J = 8.0 Hz, 2 H). The product was converted to its sodium salt as described for **26**: mp 230-240 °C. Anal. ($C_{25}H_{23}N_2NaO_5S\cdot2H_2O$) C, H, N.

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Affinity of Human Growth Hormone-Releasing Factor (1-29)NH_2 Analogues for GRF Binding Sites in Rat Adenopituitary[1]

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Previous research on growth hormone-releasing factor analogues has used pituitary cell culture assay systems to evaluate in vitro their biological activity. However, binding assay systems in which receptor affinity and peptide stability can be assessed independently have been lacking so far. Since we have recently develop a sensitive GRF binding assay with [^{125}I-Tyr^{10}]hGRF(1-44)NH_2, this method was applied to structure-affinity studies as a first step of screening GRF analogues. Acylation of the N-terminus of hGRF(1-29)NH_2 generally decreased its affinity (relative affinity to hGRF(1-29)NH_2 (RA), 26-85%). Replacement of the C-terminal carboxamide by a free carboxylic function decreased affinity likely by diminishing its proteolytic stability (RA, 57%). Removal of Tyr^1, Ser^9, Lys^{12}, Val^{13}, Gly^{15}, Gln^{16}, or Lys^{21} drastically decreased its affinity (RA, <3%). Multiple amino acid deletions in the segment 13-21 of hGRF(1-29)NH_2 also led to a loss of affinity as did replacing segment 13-15, 16-18, or 19-21 by an octanoyl moiety (RA, <1%). Removal of Asn^3, Gln^{24}, Asp^{25}, Ile^{26}, Met^{27}, and Ser^{28} or Arg^{29} had less effect on GRF receptor affinity (RA, 5-33%). Removal of Met^{27} or Ser^{28} only slightly affected hGRF(1-29)NH_2 affinity (RA, 62-78%). Altogether, these results indicate that the amino acids contained in the segment 13-21 are more important than those of 24-29 to insure high affinity receptor binding or to maintain an optimal conformation to allow GRF binding.

Introduction

Since the isolation and characterization of growth hormone (GH)-releasing factor (GRF),[2] a number of GRF analogues have been synthesized. Most[3-12] were designed to be potent agonists with potential clinical and zootechnical applications. Their structures were based upon that of hGRF(1-29)NH_2, the N-terminal portion of hGRF(1-44)NH_2, as this portion retains the full potency of the native 44 amino acid peptide to induce GH secretion in vitro and/or in vivo, in various species. Human GRF(1-29)NH_2 also possesses a high degree of sequence homology with porcine, bovine, and ovine GRF(1-29)NH_2 (≥93%) suggesting multiple applications, in various species, for a sole analogue.

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(1) Symbols and abbreviations are in accord with the recommendations of the IUPAC-IUB Commission on Biochemical Nomenclature (*Eur. J. Biochem.* 1984, *158*, 9-37). All optically active amino acids are of the L configuration, unless otherwise specified. Additional abbreviations used are as follows: Ac, acetyl; desaminoTyr, 3-(4-hydroxyphenyl)propionic acid.

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Euglycemic and Hypolipidemic Activity of PAT5A: A Unique Thiazolidinedione With Weak Peroxisome Proliferator Activated Receptor Gamma Activity

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The euglycemic and hypolipidemic activities of PAT5A, a novel pyridine analog of thiazolidinedione, have been evaluated in different animal models. Administration of PAT5A to db/db mice resulted in dose-dependent decreases in plasma glucose, triglyceride, and insulin levels, and an improved glucose tolerance. The glucose-lowering activity of PAT5A was better than that of troglitazone and comparable to that of rosiglitazone. In addition, PAT5A showed better lipid-lowering activity than troglitazone or rosiglitazone. A similar profile was seen in ob/ob mice. In high-fat–fed Sprague Dawley rats, PAT5A treatment reduced plasma triglyceride and total cholesterol levels. An in vitro peroxisome proliferator activated receptor gamma (PPARγ) transactivation assay in HEK-293 cells showed poor transactivation for PAT5A compared with rosiglitazone. PAT5A did not show any PPARα- or PPARδ-activating properties. Ex vivo study in db/db mice treated with PAT5A showed decreased activity of liver glucose 6-phosphatase, a key enzyme in gluconeogenesis. A 28-day probe toxicity study in Wistar rats did not show any treatment-related alterations in hematologic and biochemical parameters, nor any macroscopic and microscopic changes in the vital organs, whereas rosiglitazone treatment increased liver and heart weights. Our results indicate that PAT5A is a potent insulin sensitizer and hypolipidemic compound with a weak PPARγ activation potential. Both in vivo and in vitro results suggest that PAT5A improves glucose kinetics and lipid levels through mechanisms not related to PPAR activation.

INSULIN RESISTANCE is a significant pathogenic factor in type 2 diabetes.[1] The thiazolidinediones (Fig 1) are a new class of oral antidiabetic agents that increase the sensitivity of target tissues to insulin in animal models and in type 2 diabetes patients.[2,3] These compounds are synthetic, high-affinity ligands for peroxisome proliferator activated receptor gamma (PPARγ), a member of the nuclear receptor superfamily which controls the expression of genes involved in lipid and carbohydrate metabolism in target tissues.[4,5] Therefore, PPARs are considered to be the molecular targets for the therapeutic actions of this class of compounds. However, the major pitfall in the PPARγ mechanism is that it is primarily expressed in adipose tissue,[6] whereas muscle is the major site of insulin-dependent glucose disposal.[7] Perhaps, apart from a PPAR-mediated mechanism, these molecules may modulate carbohydrate and lipid metabolism through other biochemical targets.

Many of the thiazolidinediones are in various stages of clinical development. However, reports on hepatotoxicity and idiosyncratic deaths associated with troglitazone treatment have validated the need for safer insulin sensitizers for the management of type 2 diabetes.[8,9] Our search for novel insulin sensitizers, without any side effects, led to the discovery of an indole derivative of thiazolidinedione[10,11] and subsequently to PAT5A.

PAT5A is a novel thiazolidinedione analog containing pyridine (5-[4-[*N*-(2-pyridyl)-(2*S*)-pyrrolidine-2-methoxy]phenylmethylene] thiazolidine-2,4-dione, maleic acid salt) as a key heteroaromatic moiety (Fig 1). The compound ameliorated hyperglycemia, hyperinsulinemia, and hypertriglyceridemia in insulin-resistant animal models, and exhibited a lipid-lowering effect in hypercholesterolemic rats. The PPAR transactivation potential and the biochemical mechanisms involved in glucose homeostasis have also been investigated.

MATERIALS AND METHODS

Materials

PAT5A, troglitazone, rosiglitazone, and WY 14,643 were synthesized by the Medicinal Chemistry division of Dr Reddy's Research Foundation (DRF) and carbacyclin was obtained from Sigma (St Louis, MO). PAT5A and the standard compounds were examined by high-performance liquid chromatography and found to be 99.5% pure.[12] The spectral data of standard compounds were comparable to reported values.

Animals

All animal experiments were approved by the DRF Animal Experimentation Ethics Committee and were in accordance with the Committee for the Purpose of Control and Supervision of Empowerment on Animals (CPCSEA), Ministry of Social Justice and Environment, Government of India. Male C57BL/KsJ-db/db and C57BL/6J-ob/ob mice were obtained at 6 weeks of age from Jackson Laboratories (Bar Harbour, ME). Sprague Dawley and Wistar rats were bred in the DRF animal house. All animals were maintained on normal laboratory chow (National Institute of Nutrition, Hyderabad, India), ad libitum water, and a 12-hours light-dark cycle. The db/db mice were used at 8 weeks and the ob/ob mice at 10 weeks of age. Sprague Dawley rats weighing 150 to 180 g were made hypercholesterolemic by feeding a high-fat diet (2% cholesterol and 1% sodium cholate mixed in normal diet) for 6 days. The 28-day probe toxicity study used Wistar rats of either sex weighing 100 to 120 g.

Drug Administration and Blood Sampling

PAT5A was administered to db/db mice by oral gavage daily for 15 days at 3-, 10-, 30-, and 100-mg/kg doses. Troglitazone and rosiglitazone were given at doses of 800 and 10 mg/kg, respectively, and the control animals received 0.25% carboxymethylcellulose (10 mL/kg). In ob/ob mice, PAT5A was administered for 14 days at 1, 3, 10, and 30 mg/kg and rosiglitazone at 3 mg/kg. In high fat–fed Sprague Dawley rats, PAT5A and rosiglitazone were given at 10 mg/kg and troglitazone at 30 mg/kg for 3 days.

Blood samples were collected in the fed state under mild ether anesthesia of the retro-orbital sinus 1 hour after drug administration on days 0, 3, 6, 9, and 15 of treatment. Plasma samples were used to

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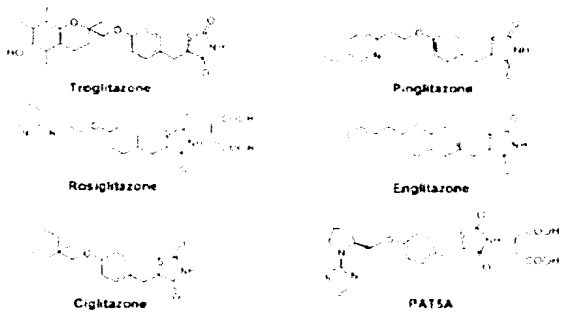

Fig 1. Structures of insulin-sensitizing thiazolidinediones and PAT5A.

estimate glucose, triglyceride, total cholesterol, and insulin levels. An oral glucose tolerance test (OGTT) was performed after 15 days of treatment in db/db mice and after 14 days in ob/ob mice. db/db mice were fasted overnight and ob/ob mice were fasted for 5 hours; the animals were challenged with 3 g/kg of glucose. Blood samples were collected at 0, 30, 60, and 120 minutes after the glucose load.

Plasmids, Transfection, and PPAR Transactivation Assay

The response element (UASGAL4 × 5) was cloned upstream of the pGL2-SV40-Luc reporter (Promega, Madison, WI), which contains the simian virus early promoter for luciferase assay. GAL4 fusions were made by fusing human PPARγ1, PPARα, or PPARδ ligand-binding domain (amino acids 174 to 475) to the C-terminal end of the yeast GAL4 DNA binding domain (amino acids 1 to 147) of the pM1 vector. PAdVantage vector (Promega) was used to enhance the luciferase expression.

HEK-293 cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum (DMEM-FBS) at 37°C in 5% CO_2. One day before transfection, cells were plated to 50% to 60% confluence in DMEM containing 10% delipidated FBS (DMEM-DFCS). Cells were transfected by means of Superfect (QIAGEN, Hilden, Germany).[15] Three hours after transfection, the reagent was removed and the cells maintained in DMEM-DFCS. Forty-two hours after transfection, cells were treated for 18 hours with phenol red free DMEM-DFCS alone or with the test compounds. The cells were lysed and assayed for luciferase activity, which was determined as fold activation relative to untreated cells using a Luciferase kit (Packard, CT) in a Packard Top Count.

Glucose 6-Phosphatase Activity in db/db Mice Liver

db/db mice were treated with PAT5A and rosiglitazone at 10 mg/kg for a period of 9 days, after which the mice were killed and liver samples collected in liquid nitrogen and stored at −80°C until further use. All procedures were performed at 0-4°C unless otherwise noted. A 10% homogenate was made in 10 mmol/L tris buffer (pH 7.5) containing 0.35 mol/L sucrose and centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected and subjected to ultracentrifugation at 100,000 × g for 1 hour. The microsomal pellet thus obtained was washed and suspended in assay buffer of 10 mmol/L Tris-HCl (pH 6.5) containing 0.25 mol/L sucrose. The final protein concentration was adjusted to 10 mg/mL.

The assay was performed in a final reaction mixture volume of 100 μL containing 50 mmol/L of glucose 6-phosphate, 100 μg of enzyme preparation, and 10 mmol/L Tris-HCl (pH 6.5). The reaction mixture was incubated at 37°C for 10 minutes and the reaction stopped by heat killing the enzyme at 80°C for 5 minutes. The glucose released by enzyme action was estimated using a kit (glucose oxidase peroxidase method). Enzyme activity is expressed in terms of units per milligram protein per minute after subtracting appropriate blanks.

Subacute Toxicity in Rats

Male and female Wistar rats were administered 100 mg/kg of PAT5A and rosiglitazone for 28 days. Animals were bled on day 14 and 28 of treatment for hematologic and biochemical estimations. After 28 days of treatment, the animals were killed, and macroscopic and microscopic examinations were performed on the major organs such as brain, liver, heart, spleen, and kidney.

Analytical Methods

Plasma glucose, triglyceride, total cholesterol, and hemoglobin were measured spectrophotometrically using commercially available kits (Dr Reddy's Laboratory, Diagnostic Division, Hyderabad, India). Plasma insulin was measured using a radioimmunoassay kit (BRIT, Mumbai, India). The hematocrit value was determined by the capillary method.

Data Analysis and Statistics

The percent reduction was calculated according to the formula:

$$1 - \frac{(TT - OT)}{(TC - OC)} \times 100,$$

where TT is test day treated; OT is zero day treated; TC is test day control; and OC is zero day control. All results are expressed as the mean ± SE of 5 experimental values. Statistical analyses were performed using analysis of variance (ANOVA) or *t* test. *P* values less than .05 were considered significant.

RESULTS

Effect of PAT5A in db/db and ob/ob Mice

Administration of PAT5A for 15 days in db/db mice at 3-, 10-, 30-, and 100-mg/kg doses decreased plasma glucose, triglyceride, and insulin levels in a dose-dependent manner (Fig 2A through C). The reduction in plasma glucose levels was observed from the third day onwards for the 10-, 30-, and 100-mg/kg doses. Plasma glucose levels reached normal values by day 6 of treatment (544 ± 15 *v* 274 ± 28, 281 ± 44, and 192 ± 32 mg/dL for control *v* 10, 30, and 100 mg/kg, respectively). The compound also significantly ($P < .05$) improved glucose tolerance (Fig 3). Troglitazone and rosiglitazone were used as standards for comparison. Troglitazone showed the maximum effect only at the 800-mg/kg dose. PAT5A and rosiglitazone at 10 mg/kg showed comparable plasma glucose- and insulin-lowering activities (50% *v* 47% for plasma glucose and 48% *v* 46% for insulin, respectively). In addition, PAT5A-treated animals showed better triglyceride-lowering activity than rosiglitazone-treated rats (77% *v* 47%, Fig 4). Animals treated with PAT5A showed a 44% improvement in glucose kinetics by OGTT as compared with 30% and 38% for rosiglitazone and troglitazone, respectively. PAT5A treatment ameliorated polydipsia but did not affect food consumption. Animals treated with rosiglitazone showed a 22% increase in body weight, whereas PAT5A-treated rats showed only a 12% increase (39.33 ± 0.99 g on day 0 and 47.75 ± 1.03 g on day 15 *v* 40.4 ± 0.6 g on day 0 and 45.4 ± 1.52 g on day 15).

In ob/ob mice, administration of PAT5A at 1, 3, 10, and 30 mg/kg showed significant ($P < .05$) dose-dependent decreases in plasma glucose, triglycerides, and insulin (Fig 5A through C). The effect of PAT5A was comparable to rosiglitazone at 3 mg/kg (53% *v* 56%, 69% *v* 41%, and 51% *v* 64% for plasma glucose, triglycerides and insulin, respectively, Fig 6). The compound treatment also showed an improvement in glucose

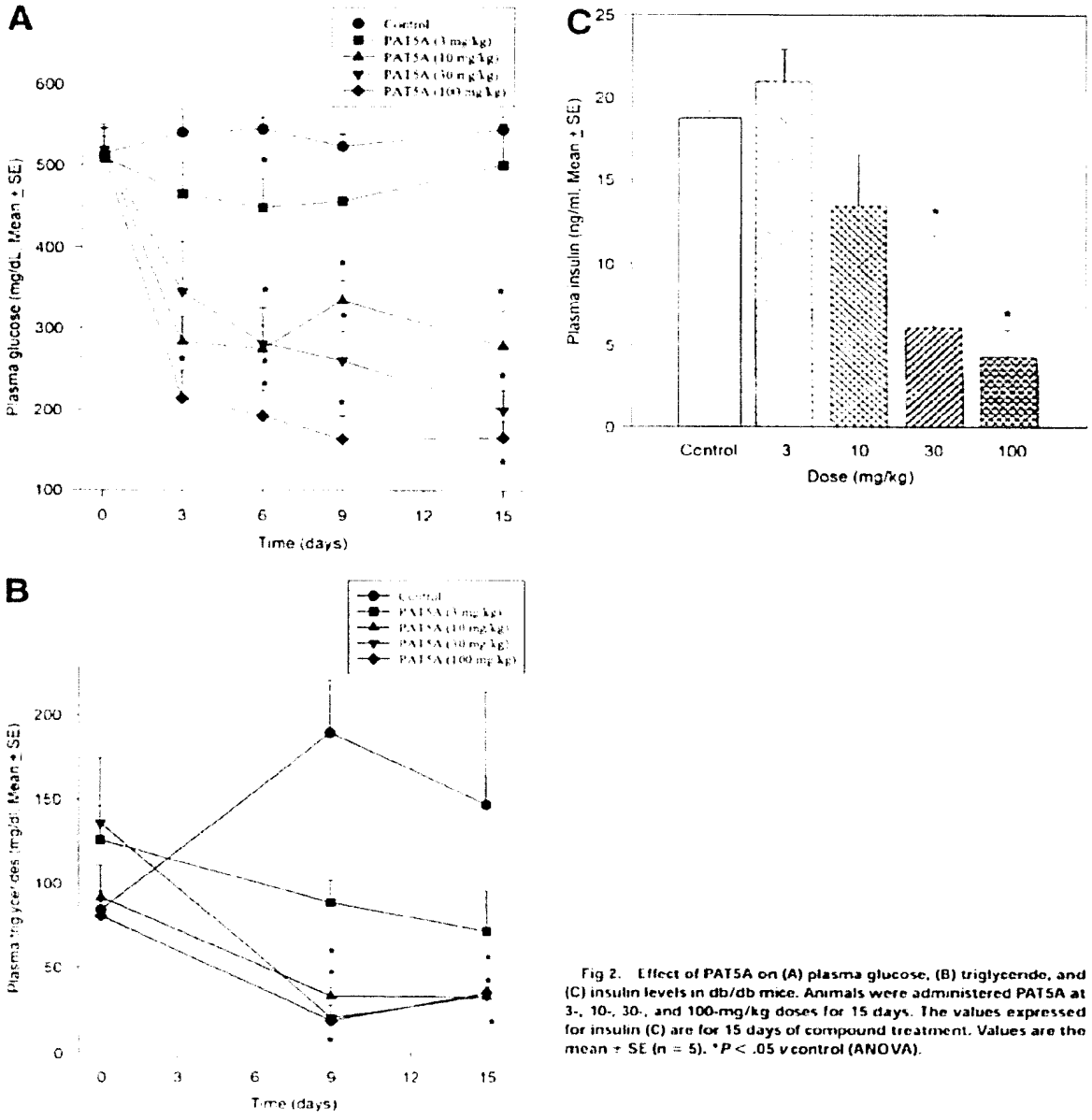

Fig 2. Effect of PAT5A on (A) plasma glucose, (B) triglyceride, and (C) insulin levels in db/db mice. Animals were administered PAT5A at 3-, 10-, 30-, and 100-mg/kg doses for 15 days. The values expressed for insulin (C) are for 15 days of compound treatment. Values are the mean ± SE (n = 5). *$P < .05$ v control (ANOVA).

tolerance in a dose-dependent manner (18%, 45%, and 54% at 3, 10, and 30 mg/kg, respectively).

Effect of PAT5A in Cholesterol-Fed Rats

To further substantiate the lipid-lowering effect seen in genetic models, a cholesterol-fed rat model was used. These animals showed a significant increase in lipid levels as compared with normal rats. PAT5A at 10 mg/kg given for 3 days significantly reduced plasma triglyceride and total cholesterol levels (Table 1). Neither rosiglitazone nor troglitazone had a significant effect on lipid levels at the 10- and 30-mg/kg doses. No change in food intake between the control and treated groups was observed.

Effect of PAT5A on PPAR Transactivation Assays

In PPARγ1 transactivation assays, PAT5A showed weak transactivation of 1- and 5-fold at 1- and 50-μmol/L concentrations, respectively (Fig 7). On the other hand, rosiglitazone showed impressive dose-dependent transactivation. Rosiglitazone at 1- and 50-μmol/L concentrations showed 12- and 17-fold transactivation. PAT5A showed only 1.5-fold activation as compared with

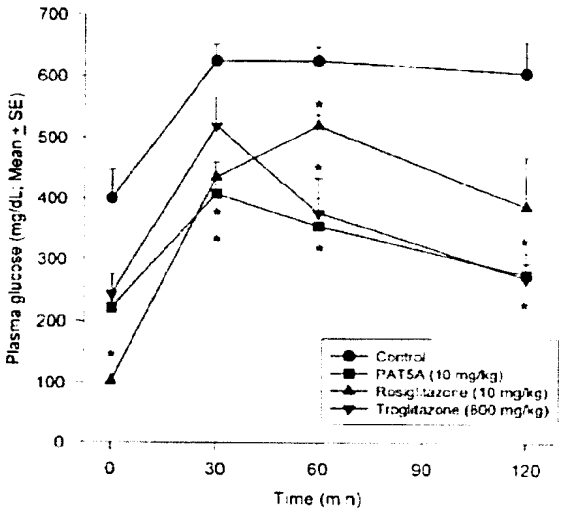

Fig 3. Comparative effect of PAT5A, rosiglitazone, and troglitazone on glucose tolerance test in db/db mice. Animals were given PAT5A and rosiglitazone at 10 mg/kg and troglitazone at 800 mg/kg for 15 days and then subjected to an oral glucose load (3 g/kg body weight) after an overnight fast. Values are the mean ± SE (n = 5). *$P < .05$ v control (ANOVA).

16-fold for WY 14,643 at a 50-μmol/L concentration in the PPARα transactivation assay. In the case of PPARδ, PAT5A showed only 1.4-fold transactivation at 50 μmol/L, whereas carbacyclin showed 10-fold transactivation at 10 μmol/L.

Effect of PAT5A on Liver Glucose 6-Phosphatase Activity

db/db mice treated with PAT5A for 9 days showed a significant decrease ($P < .05$) in liver glucose 6-phosphatase activity (Table 2). PAT5A exhibited 9-fold inhibition in activity as compared with rosiglitazone (4-fold).

Effect on Subacute Toxicity in Rats

The subacute toxicity dose for PAT5A was selected on the basis of the effective dose 50 (ED_{50}) value (5.5 mg/kg) in db/db mice. Throughout the study, animals administered with 100 mg/kg of the compound did not show any adverse behavioral changes, cumulative toxicity, or change in body weight or food consumption. No treatment-related alterations in hematologic or biochemical parameters occurred either during or at the end of the study (Table 3). No macroscopic or microscopic changes were observed for heart, liver, and bone marrow-sternum. To keep parity with PAT5A, a similar dose was selected for rosiglitazone, even though the ED_{50} value is 8.6 mg/kg. Rosiglitazone-treated animals did not show any behavioral changes or alterations in body weight. However, a significant decrease ($P < .05$) in hemoglobin and packed cell volume, and a significant increase ($P < .05$) in heart weight, were observed in rosiglitazone-treated animals.

DISCUSSION

PAT5A, a novel pyridine analog of thiazolidinedione, exhibits hypoglycemic and hypolipidemic effects in genetically diabetic C57BL/KsJ-db/db and C57BL 6J-ob/ob mice models. During early postnatal development, these animals exhibit hyperinsulinemia, hyperphagia, and obesity, which later lead to abnormalities in glucose and lipid metabolism.[14] The pathophysiology and the onset of diabetes in these models closely parallel the temporal sequence of biochemical and pathophysiologic changes in type 2 diabetes. Therefore, these models are suitable for predicting the therapeutic benefit of thiazolidinediones in these patients.[15,16] PAT5A, when administered orally to db/db mice for 15 days, dose-dependently lowered the plasma glucose and triglyceride levels by sensitization of target tissues to the action of insulin. The concomitant decrease in plasma insulin and suppression of hepatic glucose output was indicative of the insulin-sensitizing mechanism of PAT5A.

Among the thiazolidinedione series of insulin sensitizers, rosiglitazone is claimed to be more potent, more efficacious, and less toxic than troglitazone.[17] We compared the antidiabetic potential of PAT5A with that of troglitazone and rosiglitazone. In db/db mice, PAT5A and rosiglitazone showed comparable glucose-lowering effects at a 10-mg/kg dose. Troglitazone showed similar activity, albeit at a higher dose. The extent of triglyceride lowering by PAT5A was 2-fold greater than that of rosiglitazone. PAT5A treatment led to a better tolerance to an oral glucose load, suggesting an improvement in insulin sensitivity in this model. A mild increase in body weight was observed in PAT5A-treated animals, but this increase was comparatively less than that in rosiglitazone-treated animals.

The efficacy of PAT5A was also tested in the ob/ob mouse, which has impaired glucose tolerance[15] resembling early stage of type 2 diabetes. Dose-dependent lowering of plasma glucose and lipid were observed after PAT5A treatment of ob/ob mice. As seen in db/db mice, the compound dose-dependently reduced plasma insulin and improved glucose kinetics by OGTT. Additionally, it restored the ability of insulin to suppress hepatic glucose output. These results clearly suggest that PAT5A exhibited hypoglycemic and hypolipidemic activity by the sensitization of target tissues to insulin action.

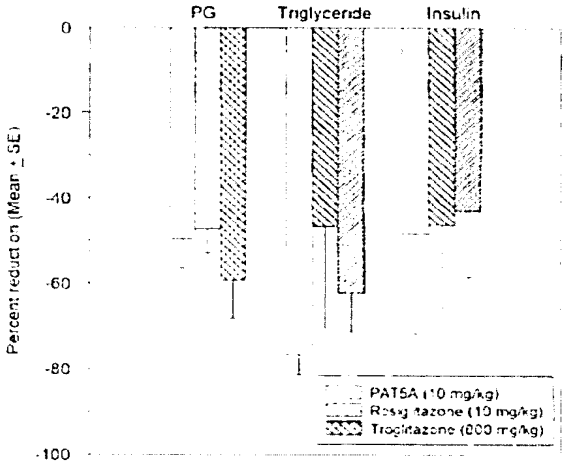

Fig 4. Effect of PAT5A, rosiglitazone, and troglitazone on plasma glucose, triglyceride, and insulin levels in db/db mice. Animals were given PAT5A and rosiglitazone at 10 mg/kg and troglitazone at 800 mg/kg for 15 days. Values are the mean ± SE (n = 5).

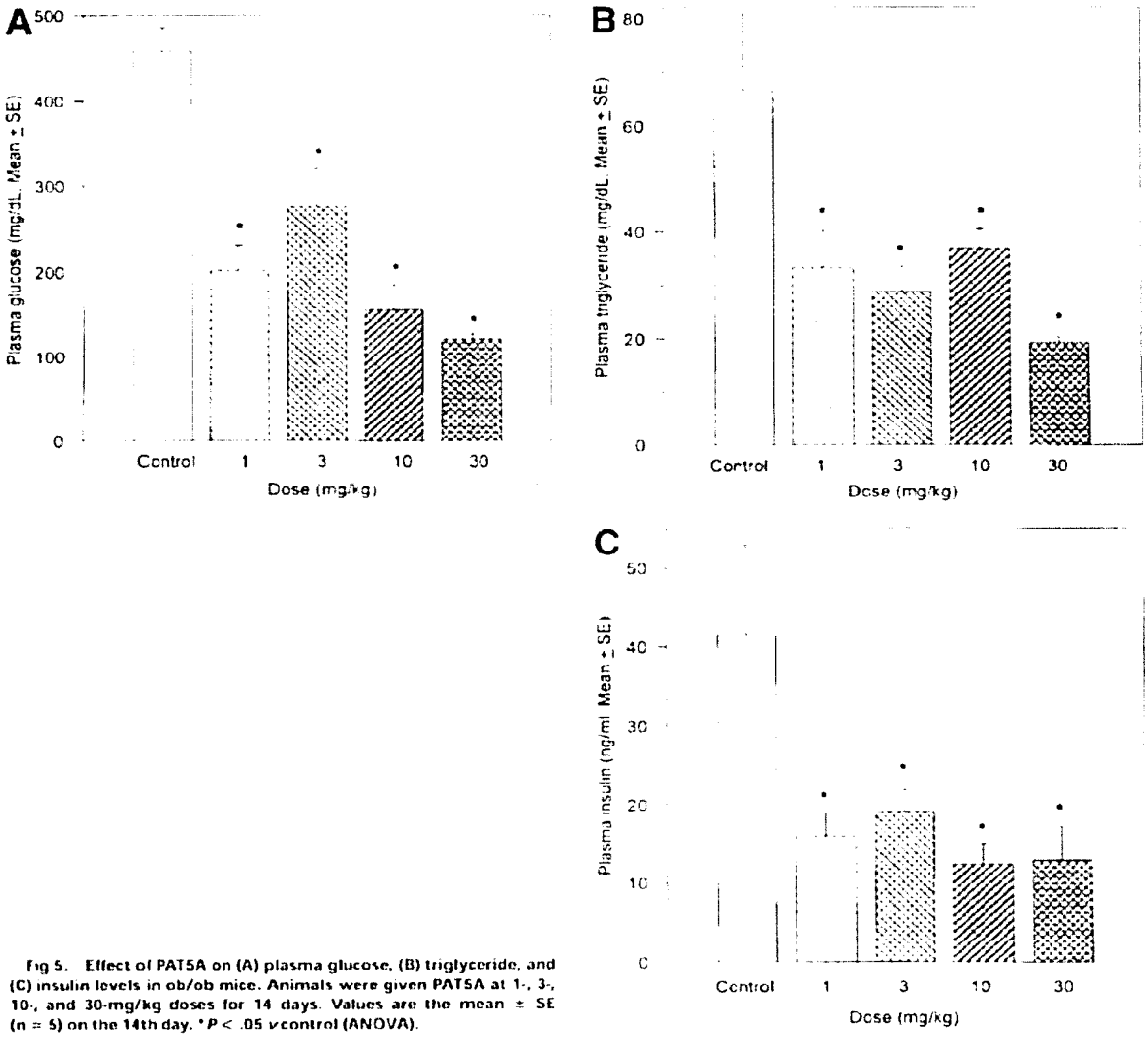

Fig 5. Effect of PAT5A on (A) plasma glucose, (B) triglyceride, and (C) insulin levels in ob/ob mice. Animals were given PAT5A at 1-, 3-, 10-, and 30-mg/kg doses for 14 days. Values are the mean ± SE (n = 5) on the 14th day. *$P < .05$ v control (ANOVA).

Although the exact molecular mechanism of action of the thiazolidinediones is not fully understood, reports indicate that they are synthetic high-affinity ligands and activators of PPARγ. The antidiabetic activity of these compounds correlates well with the order of potency for PPARγ activation.[17] In our study, the in vivo potency of PAT5A is equal to that of rosiglitazone, yet the transactivation potential is less. The interaction of PAT5A with other PPAR isoforms was ruled out, as it did not show any activation of PPARα or PPARδ.

The poor correlation between in vivo and in vitro activity of PAT5A indicates that a partial PPARγ agonism might be sufficient for its antidiabetic activity. A single-dose in vivo pharmacokinetic study conducted at a 30-mg/kg dose level in Wistar rats rules out the role of metabolites in PAT5A action, as no major metabolite was detectable in plasma.[17] This raises the possibility that PAT5A may exert its antidiabetic activity through mechanism(s) other than PPARγ activation.

Our ex vivo results from db/db mice treated with PAT5A clearly demonstrate several-fold inhibition in liver glucose 6-phosphatase activity compared with rosiglitazone. It is clear that the control of hepatic glucose output might be through the inhibition of the gluconeogenic pathway. Further studies are needed to understand the detailed mechanism of action of PAT5A.

Coronary artery disease as a result of premature atherosclerosis is a major cause of death in both type 1 and type 2 diabetes patients.[21] Although the exact cause is not fully understood, several independent risk factors may contribute, including hypercholesterolemia, hypertriglyceridemia, and hypertension.[22-24] Abnormalities in circulating lipid levels may lead to the insensitivity of peripheral tissues to insulin and glucose

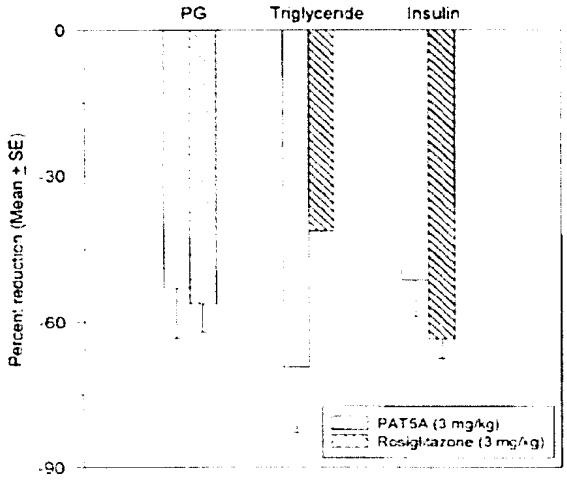

Fig 6. Effect of PAT5A and rosiglitazone on plasma glucose, triglyceride, and insulin levels in ob/ob mice. Animals were given PAT5A and rosiglitazone at 3 mg/kg for 14 days. Values are the mean ± SE (n = 5).

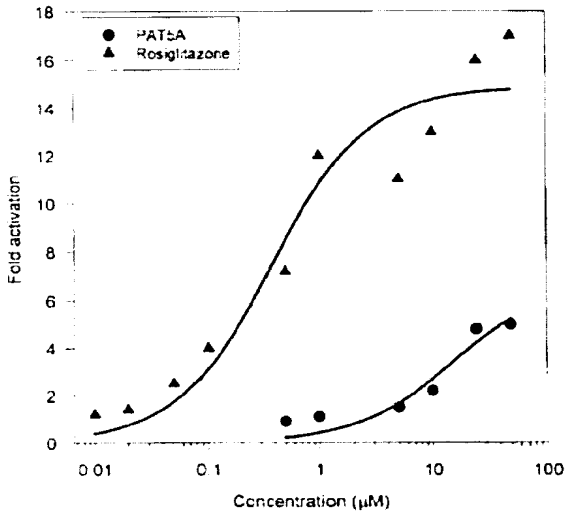

Fig 7. Activation of PPARγ by PAT5A and rosiglitazone. HEK-293 cells were transfected with Gal4-PPARγ1-LBD, pGL2(Gal4 × 5)-SV40-Luc reporter construct and PAdVantage. Luciferase activity was plotted as fold activation relative to untreated cells. Values are an average of 3 experiments conducted in triplicate.

intolerance.[25] It is advantageous to develop an insulin sensitizer with additional lipid-lowering properties for clinical use. Studies in db/db and ob/ob mice reveal the lipid-lowering potential of PAT5A. To substantiate this finding, a study was conducted in high-fat–fed Sprague Dawley rats,[26,27] with rosiglitazone and troglitazone used as standards. PAT5A-treated animals showed a significant decrease in plasma triglyceride and total cholesterol levels, whereas rosiglitazone- and troglitazone-treated animals did not show any effect. The exact mechanism of lipid-lowering by PAT5A in the genetic and high-fat–fed animals is not clearly understood, but could be a combined effect on the inhibition of lipolysis in adipose tissue, or a decreased output of triglyceride from the liver and increased clearance of the secreted triglyceride particles from the systemic circulation.

Adverse effects such as hepatotoxicity, cardiomegaly, and hemotoxicity were responsible for the cessation of development of several thiazolidinedione analogs. To assess the toxicity of PAT5A, a subacute study was conducted in Wistar rats and compared with rosiglitazone. PAT5A at 20 times the ED_{50} dose did not show any treatment-related adverse effects on hemoglobin, packed cell volume, or liver and heart weight. The compound treatment did not induce any macroscopic or microscopic changes in these animals. Rosiglitazone at a similar dose significantly decreased hemoglobin and packed cell volume. In Wistar rats, a single-dose pharmacokinetics study conducted with PAT5A and rosiglitazone showed linear pharmacokinetics at 3-, 10-, and 30-mg/kg doses (data not given). Similarly, in db/db mice, the dose-dependent decrease in plasma glucose levels after PAT5A treatment is indicative of linear pharmacokinetics. It is reasonable to believe that both compounds may have comparable plasma levels at 100 mg/kg. The PAT5A-treated animals did not show any treatment-related adverse effects compared with rosiglitazone-treated animals, suggesting that the toxicity may be associated with the PPARγ mechanism

Table 1. Effect of PAT5A, Rosiglitazone, and Troglitazone in High-Fat–Fed Sprague Dawley Rats

Group	TG (mg/dL)	TC (mg/dL)
Normal-diet control	36.82 ± 2.32	113.19 ± 4.18
High-fat–fed control	316.80 ± 24.70	697.50 ± 48.90
PAT5A	141.40 ± 16.90*	438.10 ± 85.60*
Rosiglitazone	255.03 ± 29.37	598.32 ± 29.52
Troglitazone	269.49 ± 41.12	857.39 ± 11.10

NOTE. Male Sprague Dawley rats weighing 150 to 180 g were made hyperlipidemic by feeding a high-fat diet containing 2% cholesterol and 1% sodium cholate for 6 days. PAT5A and rosiglitazone were given at 10 mg/kg and troglitazone was given at 30 mg/kg for 3 days. The high-fat diet was continued during the treatment period. Values are the mean ± SE (n = 4 to 16).

*$P < .05$ v high-fat–fed control (ANOVA).

Abbreviations: TG, triglyceride; TC, total cholesterol.

Table 2. Effect of PAT5A and Rosiglitazone on Liver Glucose 6-Phosphatase Activity in C57BL/KsJ-db/db Mice

Treatment Group	Glucose 6-Phosphatase (U/mg protein)
Control	1.48 ± 0.22
PAT5A	0.16 ± 0.14*
Rosiglitazone	0.37 ± 0.16

NOTE. PAT5A and rosiglitazone were given at 10 mg/kg per dose for 9 days. Values are the mean ± SE (n = 5). One unit of enzyme will release 1 μmol of glucose from glucose 6-phosphate under the assay conditions.

*$P < .05$ v control (*t* test).

Table 3. Effect of PAT5A and Rosiglitazone in Subacute Toxicity in Rats

Parameter	PAT5A	Rosiglitazone
Hematology		
Hemoglobin	NE	−11*
Packed cell volume	NE	−9*
Organ weight		
Heart	NE	+22*
Liver	NE	+23

NOTE. Male and female Wistar rats weighing 100 to 120 g were orally administered PAT5A and rosiglitazone at 100 mg/kg per dose for 28 days. The rats were killed on day 29 and blood collected for hematologic and biochemical estimations. Brain, heart, liver, spleen, kidney, and other vital organs were examined both macroscopically and microscopically. Values are expressed as percent change (n = 10).

*$P < .05$ v control (ANOVA).

Abbreviation: NE, No effect.

rather than the chemical class effect. These encouraging results gave us impetus to study PAT5A further.

In summary, PAT5A is a novel thiazolidinedione that ameliorates hyperglycemia, hyperinsulinemia, and dyslipidemia in different animal models. The overall antidiabetic potency of PAT5A is much better than that of troglitazone and comparable to that of rosiglitazone. Hyperglycemia was controlled through an inhibitory effect on glucose 6-phosphatase, a key enzyme in gluconeogenesis. Although the compound showed good in vivo activity, it is mechanistically distinct from the other thiazolidinediones in that it had weak PPARγ transactivation potential. A preliminary toxicity study did not show any adverse effects. In conclusion, PAT5A is an effective and safe novel thiazolidinedione for the treatment of diabetes and related disorders in type 2 diabetes. With the initial interesting results, further studies are being performed to understand the mechanism of action of this compound.

ACKNOWLEDGMENT

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Reduction of Insulin Resistance in Obese and/or Diabetic Animals by 5-[4-(1-Methylcyclohexylmethoxy)benzyl]-thiazolidine-2,4-dione (ADD-3878, U-63,287, Ciglitazone), a New Antidiabetic Agent

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SUMMARY

Effects of 5-[4-(1-methylcyclohexylmethoxy)benzyl]-thiazolidine-2,4-dione (ADD-3878, U-63,287, Ciglitazone) on glucose and lipid metabolism were examined in various animal models. ADD-3878, administered as a dietary admixture (30–186 mg/kg/day) to obese-diabetic yellow KK (KK-A^y) mice, markedly suppressed the diabetic syndromes (hyperglycemia, hypertriglyceridemia, and hyperinsulinemia), accompanied by the reduction of insulin resistance as manifested by improvement of overall insulin sensitivity in either the insulin tolerance test or the steady-state blood glucose test. Chronic administration of ADD-3878 for as long as 12 wk to young yellow KK mice, which were in the early stage of diabetes and obesity, depressed age-dependent rises in blood glucose, plasma triglyceride, and insulin without exerting any effect on body weight.

When orally administered to obese Zucker-fatty rats, ADD-3878 decreased plasma insulin and triglyceride in a dose-dependent manner (5–100 mg/kg/day). The treated rats showed increased tolerance and decreased insulin secretion in response to oral glucose. The glycemic response to insulin and the steady-state plasma glucose were also normalized in the treated rats. Chronic administration of ADD-3878 to young fatty rats for as long as 12 wk decreased the dose-dependent rises in blood glucose, plasma triglyceride, and insulin without exerting any effect on body weight.

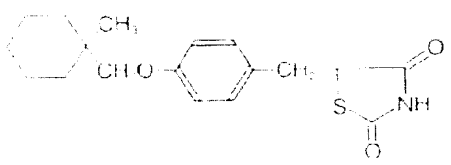
ADD-3878 had no effect on glucose and lipid metabolism of young Sprague-Dawley rats and mild streptozotocin-diabetic rats. However, in old Sprague-Dawley rats that were moderately insulin resistant and hyperlipidemic compared with young ones, ADD-3878 decreased plasma triglyceride and insulin and improved insulin sensitivity.

Five-day administration of ADD-3878 to beagle dogs with slightly impaired glucose tolerance increased glucose tolerance and suppressed postprandial rises in plasma glucose, insulin, and triglyceride.

Based on these results, ADD-3878 is effective on abnormal glucose and lipid metabolism associated with insulin resistance or obesity through reduction of peripheral insulin resistance. Therefore, ADD-3878 is expected to be useful in the treatment of hyperglycemia, hyperinsulinemia, and hyperlipemia in obese type II diabetes and obesity. DIABETES 32:804–810, September 1983.

Insulin resistance is a characteristic feature of both type II diabetes mellitus and obesity and is frequently associated with hyperinsulinemia and hyperlipidemia.[1-4] Although the mechanism of insulin resistance is still obscure, there is evidence that insulin resistance or decreased efficiency of insulin action is involved in the pathogenesis of type II diabetes and obesity in human subjects[5-8] and animal models.[9-11] However, few drugs are developed on the basis of this concept, although some evidence suggests that some sulfonylureas potentiate insulin action in peripheral tissue by increasing the number of insulin receptors.[12-15]

We have been looking for new compounds that decrease insulin resistance in diabetic and/or obese animals. On the basis of pharmacologic findings, ADD-3878 {5-[4-(1-methylcyclohexylmethoxy)benzyl]thiazolidine-2,4-dione} has been selected as one of the promising candidates. The present article describes unique pharmacologic actions of this compound on insulin resistance and carbohydrate and lipid metabolism in various insulin-resistant animal models. (The chemical structure of ADD-3878 is given below.)



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MATERIALS AND METHODS

Animals. Yellow KK (KK-*A*^{y}/+) mice were bred by mating female KK mice with male yellow KK mice as reported previously.[19] Zucker-fatty (*fa/fa*) rats were bred by matings between heterozygous lean rats (*fa*/+). They were produced in our Drug Safety Evaluation Laboratories of this division. Sprague-Dawley rats were purchased from CLEA Japan Inc. These mice and rats were maintained on a laboratory chow, CE-2 (CLEA, Japan), consisting of 52.7% carbohydrate, 23.6% protein, 4.4% fat, 4.9% fiber, 6.6% minerals and vitamins, and water ad libitum. They were housed in individual metal cages in a room controlled for temperature (23 ± 1°C), humidity (55 ± 5%), and light (0800–2000 h).

Male Sprague-Dawley rats (6 wk old) were rendered diabetic by intravenous injection of streptozotocin (40 mg/kg × 2 with a 6-day interval) in citrated normal saline prepared immediately before injection. The diabetic rats, which exhibited hyperglycemia (>350 mg/dl) in the fed state but normoglycemia in a 20-h fast, were selected for use in the experiment 20 days after the injection of streptozotocin.

Five male beagle dogs (13–14 kg, 1–3 yr old), whose oral glucose tolerance was slightly lower than the normal range, were selected from 24 dogs supplied by CLEA Japan Inc. They were fed a CD-5 diet (CLEA, Japan) consisting of 48.6% carbohydrate, 25.2% protein, 6.0% fat, 5.5% fiber, and 8.7% minerals and vitamins at 0830–0930 h.

Drug administration. ADD-3878 was synthesized as reported previously.[16] To adult rats and dogs, the compound suspended in 5% gum arabic solution was orally administered by stomach tube. To mice and young fatty rats, the compound was given as a dietary admixture in CE-2 powdered diet. The dosages were estimated from the diet intake. In the case of yellow KK mice, the diet intake was determined as total food consumption of all mice in a group.

Glucose and insulin tolerance tests in rats and mice. In the oral glucose tolerance test, rats received a 40% glucose solution (2 g glucose/kg) after 20 h of fasting. In the insulin tolerance test, rats and mice were fasted for 20 h and received ip insulin (Novo, regular, 0.5 or 1.0 U/kg) (Novo Industries, Copenhagen, Denmark).

Intravenous glucose tolerance test (IV-GTT) in dogs. IV-GTT was performed according to the method of Haneda et al.[17] After 24 h of fasting, dogs were subjected to IV-GTT with a rapid injection (20 s) of 0.5 g/kg glucose into the foreleg vein. Blood samples were drawn 5, 10, 20, and 30 min after glucose injection for measurement of plasma glucose and insulin.

Steady-state plasma glucose (SSPG). The SSPG of fatty rats was measured by the method of Shen et al.[18] After 20 h of fasting, the rats received a bolus injection of propranolol (700 μg/rat) followed by a constant infusion of glucose (29 mg/kg/min), insulin (20 mU/kg/min), epinephrine (0.4 μg/kg/min), and propranolol (20 μg/kg/min) into the jugular vein. The mean SSPG and steady-state plasma insulin (SSPI) were determined with the values obtained from the blood samples drawn 90, 120, and 150 min after the start of the infusion.

For the measurement of steady-state blood glucose (SSBG) of yellow KK mice, the simplified method previously reported by Taketomi et al.[14] was used. After 20 h of fasting, mice were subcutaneously injected with epinephrine (100 μg/kg), propranolol (5 mg/kg), glucose (3 g/kg, [3-^{3}H]-glucose, 1.36 μCi/mouse), and insulin (0, 1, or 2 U/kg). Blood samples were obtained from the orbital sinus 45, 60, and 75 min after the injection for determination of mean SSBG and SSPI values and specific activity of blood glucose.

Estimation of peripheral disposal of exogenous triglyceride. The removal rate of plasma triglyceride was determined according to the method of Boberg et al.[12] Fatty rats were injected with Intralipid emulsion (10%, 3 ml/kg) via the tail vein. Plasma triglyceride concentration was measured 10, 20, and 30 min after the injection to calculate the lipid disappearance rate.

Estimation of hepatic triglyceride secretion. The hepatic triglyceride secretion rate was determined according to the method of Otway and Robinson.[13] Fatty rats were injected with Triton WR-1339 (20% in saline, 500 mg/kg) via the tail vein. Plasma triglyceride concentration was measured 0, 15, 30, and 45 min after the injection. The rate of hepatic triglyceride output was manifested as the increment of plasma triglyceride concentration per minute.

Analytic methods. Blood samples were deproteinized with Ba(OH)$_2$ and ZnSO$_4$ solutions. After centrifugation, the resultant supernatant was used for the determination of glucose, which was determined by a glucose-oxidase method.[21] For determination of the specific activity of blood glucose, an aliquot of the supernatant was dried to remove tritiated water and the resultant precipitate was resolved in 0.5 ml of

TABLE 1
Effects of ADD-3878 on body weight, food intake, blood glucose, plasma insulin, and plasma lipids in male and female yellow KK mice

	Dosage (mg/kg/day)	Body weight (g)	Food intake (g/4 days)	Blood glucose (mg/dl)	Plasma insulin (μU/ml)	Plasma NEFA (μeq/l)	Plasma triglyceride (mg/dl)	Plasma cholesterol (mg/dl)	Plasma phospholipid (mg/dl)
Male									
Control		35.0 ± 0.8	76.3	420 ± 63	1268 ± 686	127 ± 13	512 ± 52	147 ± 11	310 ± 31
ADD-3878	39	36.4 ± 1.3	77.5	278 ± 104‡	1098 ± 834	86 ± 29†	344 ± 164*	130 ± 15	290 ± 30
ADD-3878	78	35.6 ± 2.0	72.0	244 ± 62§	497 ± 274†	52* ± 17§	314 ± 79§	114 ± 16‡	263 ± 33*
ADD-3878	156	36.4 ± 0.8	76.7	183 ± 17§	383 ± 229†	29 ± 3‡	266 ± 34§	96 ± 6§	235 ± 12§
Female									
Control		40.3 ± 2.1	85.2	415 ± 80	1393 ± 627	155 ± 37	539 ± 108	227 ± 37	394 ± 66
ADD-3878	30	39.8 ± 1.2	83.6	272 ± 64‡	942 ± 504	113 ± 44	373 ± 69‡	229 ± 37	393 ± 50
ADD-3878	74	40.2 ± 1.1	83.4	216 ± 32§	562 ± 534	63 ± 33‡	291 ± 76§	180 ± 46	357 ± 59
ADD-3878	155	39.9 ± 1.7	84.3	210 ± 13§	88 ± 62§	95 ± 37†	266 ± 65§	177 ± 21‡	313 ± 48*

ADD-3878 was given to yellow KK mice (M = 6, F = 5) as dietary admixture for 4 days. Body weight, food intake, blood glucose, and plasma lipids were determined in the morning (ad lib). *P < 0.05, †P < 0.02, ‡P < 0.01, §P < 0.001 versus control. Mean ± SE (M = 6).

TABLE 2
Effects of chronic administration of ADD-3878 on body weight, blood glucose, plasma insulin, and plasma triglyceride in young yellow KK mice and fatty rats

		Yellow KK mice (no. of animals)		Fatty rats (no. of animals)	
	Weeks of treatment	Control 10	ADD-3878 10	Control 8	ADD-3878 8
Body weight (g)	2	29.0 ± 1.2	28.6 ± 1.5	211 ± 18	204 ± 26
	4	34.1 ± 1.0	34.4 ± 1.3	314 ± 21	311 ± 35
	8	39.7 ± 0.6	39.5 ± 1.6	448 ± 25	466 ± 41
	12	42.0 ± 1.3	42.7 ± 2.0	517 ± 27	550 ± 48
Food intake (g/wk)	2	42.8	41.4	181 ± 15	185 ± 20
	4	36.0	38.2	215 ± 23	232 ± 22
	8	40.2	38.9	211 ± 28	238 ± 28
	12	36.4	39.7	175 ± 20	175 ± 18
Blood glucose (mg/dl)	2	191 ± 58	171 ± 10	91 ± 7	87 ± 9
	4	327 ± 73	196 ± 53‡	105 ± 41	81 ± 9
	8	429 ± 35	312 ± 59‡	136 ± 51	90 ± 8*
	12	442 ± 68	311 ± 94†	136 ± 51	98 ± 20
Plasma insulin (μU/ml)	2	—	—	510 ± 304	153 ± 65†
	4	1250 ± 42*	347 ± 216‡	1029 ± 560	305 ± 110†
	8	4330 ± 2020	2180 ± 759†	2103 ± 599	631 ± 142‡
	12	6520 ± 2030	4290 ± 2130*	2550 ± 568	790 ± 419‡
Plasma triglyceride (mg/dl)	2		—	197 ± 40	137 ± 39†
	4	—	—	384 ± 129	106 ± 34‡
	8	—	—	667 ± 223	300 ± 183†
	12	449 ± 129	322 ± 51†	592 ± 332	282 ± 146*

Female yellow KK mice (4 wk old) and male fatty rats (4 wk old) were kept on the CE-2 diet with or without ADD-3878 (0.05%) for 12 wk. Body weight, food intake, blood glucose, and plasma insulin and triglyceride were determined the next morning after each dosing. *P < 0.05, †P < 0.01, ‡P < 0.001 versus control. Mean ± SD.

water. Radioactivity of glucose was determined in a liquid scintillation spectrometer (Aloka LSC-903, Aloka Co. Ltd., Japan) using Bray's cocktail.[22] Plasma immunoreactive insulin was measured by the double-antibody method based on Hales and Randle[23] using a commercial kit (Amersham, England) with human insulin as a standard. Plasma triglyceride, cholesterol, phospholipid, and NEFA were determined using commercially available assay kits (Wako Chemicals, Japan).

Reagents. L-Epinephrine from E. Merck, A. G. (Darmstadt, Germany), DL-propranolol hydrochloride from Sigma Chemical Company (St. Louis, Missouri), and Novo regular insulin from Novo Laboratories were used. [3-^{3}H]Glucose was purchased from New England Nuclear Corp. (Boston, Massachusetts).

Evaluation of the data. The data were presented by mean ± SD and statistically analyzed by Student's *t* test. The minimum effective dose of ADD-3878 was defined as the smallest dose to produce a statistically significant decrease ($P < 0.05$, Student's *t* test) in blood glucose and lipid concentration as compared with those of the control group.

RESULTS

EFFECTS OF ADD-3878 ON GLUCOSE AND LIPID METABOLISM IN GENETICALLY DIABETIC AND OBESE MICE, YELLOW KK

Yellow KK mice have genetically determined obese and diabetic syndromes such as hyperglycemia, hyperinsulinemia, hypertriglyceridemia, and severe insulin resistance, all of which increase with age.[19]

Effects on hyperglycemia and hyperlipidemia. When ADD-3878 was given to male or female yellow KK mice (9 wk old) as a dietary admixture (30–186 mg/kg/day, calculated from food intake) for 4 days, nonfasting blood glucose, plasma insulin, and plasma lipids, especially triglyceride, were decreased in a dose-dependent manner, but neither

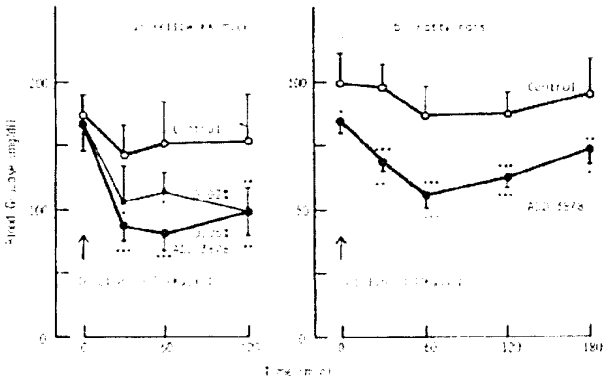

FIGURE 1. Effects of ADD-3878 on insulin tolerance in yellow KK mice and fatty rats. (a) Male yellow KK mice (9 wk old) were kept on the CE-2 diet with ADD-3878 (0.02% or 0.05%) for 4 days. They were given insulin (0.5 U/kg, i.p.) on day 5 after 20 h of fasting. (b) ADD-3878 (50 mg/kg/day) was orally administered to male fatty rats (12 wk old) for 8 days. The rats were given insulin (1 U/kg, i.p.) on day 9 after 20 h of fasting. *P < 0.05, **P < 0.01, ***P < 0.001 versus control. †P < 0.05, ††P < 0.01, †††P < 0.001 versus initial. Mean ± SD (N = 5).

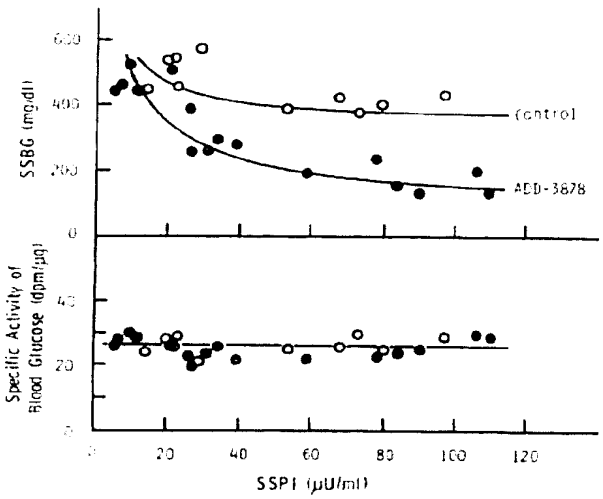

FIGURE 2. Relationship between SSPI and SSBG or the specific activity of blood glucose in yellow KK mice. Male yellow KK mice (12 wk old) were kept on the CE-2 diet with or without ADD-3878 (0.15%) for 4 days. After 20 h of fasting, they were subcutaneously injected with epinephrine (100 μg/kg), propranolol (5 mg/kg), glucose (3 g/kg, [3-^{3}H]glucose, 1.36 μCi/mouse), and insulin (0, 1, or 2 U/kg). Details of procedures are described in MATERIALS AND METHODS.

food intake nor body weight was altered (Table 1). A significant fall of blood glucose (by more than 10%) could be detected at a dose as low as 7 mg/kg/day (data not shown). When ADD-3878 was chronically administered (12 wk) to young yellow KK mice (4 wk old), which were in the early phase of obesity and diabetes, age-dependent increases in blood glucose, plasma insulin, and triglyceride were markedly suppressed (Table 2). However, body weight and food intake were not affected throughout the experiment. A single oral administration of ADD-3878 (100 mg/kg) resulted in no changes in blood glucose, plasma insulin, and triglyceride (data not shown).

Effects on insulin resistance. Insulin resistance of yellow KK mice was manifested by an impaired glycemic response to exogenous insulin as high as 0.5 U/kg. The glycemic response was improved dose-dependently after a 4-day treatment with this compound (Figure 1a). To clarify the effect of ADD-3878 on peripheral insulin resistance, the steady-state blood glucose (SSBG) was determined at different steady-state plasma insulin (SSPI) levels (Figure 2). In accordance with results obtained in KK and C57BL/6 mice as previously reported,[8] specific activity of blood glucose of control and treated mice did not change with increasing SSPI, suggesting no differences in exogenous glucose influx and hepatic glucose output among the animals. Therefore, SSBG could indicate relative impedance of glucose metabolism of the peripheral tissue under this condition. The SSBG at 20 μU/ml of SSPI was about 500 mg/dl and this value indicated severe insulin resistance of these mice (cf. 160 mg/dl for C57BL mice[18]). SSBG of control and treated groups decreased gradually with increasing levels of SSPI. The SSBG response to SSPI was exaggerated in ADD-3878-treated mice (Figure 2). These findings indicate that ADD-3878 decreased peripheral insulin resistance in yellow KK mice.

EFFECTS OF ADD-3878 ON GLUCOSE AND LIPID METABOLISM IN GENETICALLY OBESE RATS, FATTY

Fatty rats develop obesity-associated syndromes such as hyperinsulinemia, hypertriglyceridemia, and insulin resistance as their genetic traits.[3]

Effects on hyperlipemia and hyperinsulinemia. When orally administered to fatty rats for 7 days, ADD-3878 (50 mg/kg/day) decreased nonfasting plasma insulin and triglyceride without changing body weight and food intake (expt. 1 in Table 3). Blood glucose, plasma cholesterol, and NEFA were also decreased by ADD-3878. In the dose-finding study, a significant reduction of plasma triglyceride (by 30%) was observed at a dose as low as 10 mg/kg/day (data not shown).

To examine the lipid-lowering mechanism of ADD-3878, the rates of lipid removal and hepatic triglyceride output were calculated by measuring plasma triglyceride after Intralipid injection and after Triton injection, respectively. In fatty rats treated with ADD-3878, the removal rate of plasma triglyceride was markedly elevated, but the hepatic output of triglyceride was not altered (expt. 3 in Table 3).

When the compound was chronically administered (12 wk) to young fatty rats (4 wk old) as a dietary admixture, the age-dependent elevation of plasma triglyceride and insulin levels was markedly suppressed (Table 2). A slight elevation of

TABLE 3
Effects of ADD-3878 on body weight, food intake, blood glucose, plasma insulin (IRI), plasma lipids, steady-state plasma glucose (SSPG), plasma triglyceride removal, and hepatic triglyceride output in fatty rats

	Control	ADD-3878
Expt. 1		
Body weight (g)	505 ± 24	522 ± 34
Food intake (g/day)	23 ± 3	25 ± 2
Blood glucose (mg/dl)	111 ± 11	79 ± 10‡
Plasma IRI (μU/ml)	2110 ± 243	897 ± 210§
Plasma triglyceride (mg/dl)	872 ± 379	286 ± 85‡
Plasma cholesterol (mg/dl)	165 ± 28	120 ± 26*
Plasma NEFA (μeq/L)	458 ± 100	249 ± 35‡
Expt. 2		
SSPG (mg/dl)	400 ± 31	325 ± 37‡
SSPI (μU/ml)	980 ± 155	785 ± 227
Expt. 3		
Plasma triglyceride removal (%/min)	3.62 ± 0.61	4.85 ± 0.63†
Hepatic triglyceride output (mg/dl/min)	21.7 ± 7	20.0 ± 3.7

Expt. 1: ADD-3878 (50 mg/kg/day) was orally administered to male fatty rats (12–14 wk old) for 7 days. Blood samples were taken 20 h after the last dosing.
Expt. 2: ADD-3878 (100 mg/kg/day) was orally administered to male fatty rats (12 wk old) for 10 days. After 20 h fasting, SSPG was determined as described in MATERIALS AND METHODS.
Expt. 3: ADD-3878 (50 mg/kg/day) was orally administered to male fatty rats (7 wk old) for 6 days. The removal rate of plasma triglyceride on day 5 was estimated from the disappearance of plasma triglyceride after injection of Intralipid. The rate of hepatic triglyceride output on day 9 was determined from increments of plasma triglyceride concentration after injection of Triton WR-1339.
*$P < 0.05$, †$P < 0.02$, ‡$P < 0.01$, §$P < 0.001$ versus control. Mean ± SD ($N = 5$).

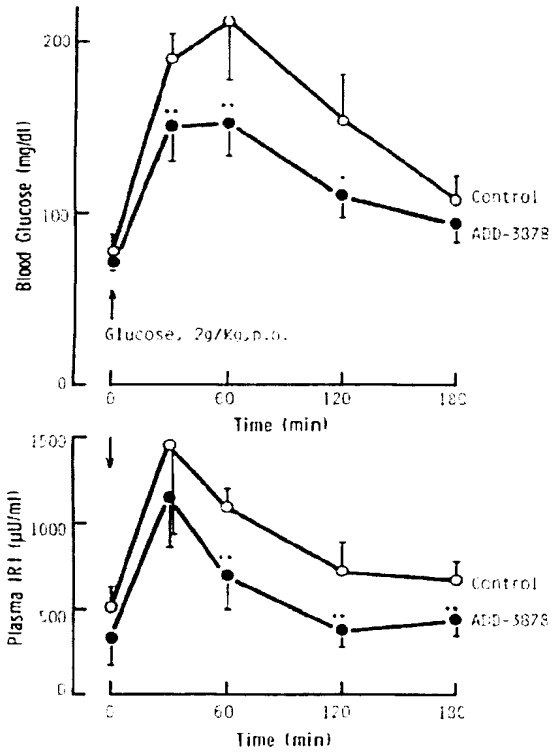

FIGURE 3. Effect of ADD-3878 on oral glucose tolerance in fatty rats. ADD-3878 (50 mg/kg/day) was orally administered to male fatty rats (14 wk old) for 8 days. After the last administration, rats were fasted for 20 h and subjected to an oral glucose tolerance test. *P < 0.02, **P < 0.01 versus control. Mean ± SD (N = 5).

blood glucose with advancing age was also depressed by the treatment (Table 2).

Effects on glucose tolerance and insulin resistance. To orally administered glucose, fatty rats exhibited marked responses of blood glucose and plasma insulin, which indicated glucose intolerance and insulin resistance (Figure 3). ADD-3878 administration resulted in marked normalization of the abnormal responses of blood glucose and plasma insulin in this test.

In the insulin tolerance test, fatty rats showed no significant change in blood glucose in response to a dose of insulin as high as 1 U/kg (Figure 1b). ADD-3878 administration decreased fasting blood glucose and increased the glycemic response to insulin.

In the SSPG study, the glucose level was significantly lower in ADD-3878-treated rats while the insulin level was somehow higher in control rats in spite of the same insulin infusion rate carried out in the both groups (expt. 2 in Table 3). If SSPI had been made the same in both groups by regulating insulin infusion, SSPG would have been much lower in the treated group.

All of these results indicate that ADD-3878 decreases the obesity-associated insulin resistance of fatty rats.

EFFECTS OF ADD-3878 ON GLUCOSE AND LIPID METABOLISM IN SPRAGUE-DAWLEY (SD) RATS AND STREPTOZOTOCIN-INDUCED-DIABETIC RATS

ADD-3878 (100 mg/kg/day, p.o.) showed no effects on blood glucose and plasma lipid in young SD rats (5 wk old), although the administration period was extended as long as 16 days (Table 4). Old SD rats (20 wk old), as compared with young ones, were moderately obese, hyperinsulinemic, and hypertriglyceridemic as described in many papers[24,25] and had decreased plasma insulin and triglyceride levels in response to ADD-3878 administration (Table 4). Furthermore, a single administration of ADD-3878 to fasted young and old rats altered neither blood glucose nor plasma insulin (data not shown).

The same extent of glycemic response to insulin was obtained in the young and old rats by using a higher dose of the hormone with old rats, indicating insulin resistance of the old rats (Figure 4). ADD-3878 administration (100 mg/kg/day) increased the response to insulin in the old rats but not in the young rats (Figure 4).

In mildly streptozotocin-diabetic rats, oral ADD-3878 administration (100 mg/kg/day, for 12 days) neither decreased blood glucose and plasma triglyceride levels nor improved glucose tolerance (data not shown).

EFFECTS OF ADD-3878 ON GLUCOSE AND LIPID METABOLISM IN BEAGLE DOGS

Beagle dogs used in the experiment were selected from normal dogs based on glucose tolerance and insulin response. They were normal in plasma glucose, triglyceride, and insulin but slightly intolerant and hyperinsulinemic in response to oral glucose. When ADD-3878 (100 mg/kg/day) was orally administered to these dogs for 5 days, the mean k values (plasma glucose clearance rate) of IV-GTT were increased from 3.8 to 4.73%/min without a significant increment of plasma insulin area, indicating increased efficiency of insulin action in glucose metabolism by the treatment with this compound. The treated dogs showed no change in fasting levels of plasma components except decreasing plasma cholesterol, but significant decreases in postprandial plasma glucose, insulin, and triglyceride levels (Table 5). The lowering effect on the postprandial plasma triglyceride was dose-dependent, with a significant marginal effect (by 20%) observed at a dose as low as 5 mg/kg body wt (data not shown).

DISCUSSION

Type II diabetes and obesity are very frequently accompanied by insulin resistance, which appears to be a direct or indirect cause for metabolic and endocrinologic abnormalities, such as hyperglycemia, hyperlipemia, and hyperinsulinemia, although its mechanism is still obscure.[1-6] In genetically obese and/or diabetic animals including yellow (obese) KK mice and fatty rats used in the present studies, obese and diabetic syndromes are accompanied with insulin resistance.[9-11]

ADD-3878, a new compound with the thiazolidine ring differing from the sulfonylureas and biguanides in its chemical structure, had a marked effect on abnormalities of glucose and lipid metabolism in yellow KK mice and fatty rats, such as hyperinsulinemia, hypertriglyceridemia, hyperglycemia,

TABLE 4
Effects of ADD-3878 on body weight, blood glucose, plasma triglyceride (TG), plasma NEFA, and plasma insulin (IRI) in normal rats

Days on treatment of ADD-3878	Young rats		Old rats	
	Control	ADD-3878	Control	ADD-3878
3				
Body weight (g)	202 ± 8	200 ± 9	635 ± 43	632 ± 38
Blood glucose (mg/dl)	108 ± 12	102 ± 5	97 ± 4	105 ± 5
Plasma TG (mg/dl)	111 ± 15	100 ± 31	324 ± 119	143 ± 45†
Plasma NEFA (μeq/L)	300 ± 61	223 ± 64	316 ± 89	577 ± 201
Plasma IRI (μU/ml)	46 ± 9	25 ± 10‡	64 ± 16	40 ± 9
8				
Body weight (g)	234 ± 9	232 ± 10	623 ± 40	615 ± 33
Blood glucose (mg/dl)	91 ± 6	92 ± 1	83 ± 2	92 ± 5
Plasma TG (mg/dl)	83 ± 8	93 ± 9	201 ± 38	109 ± 13§
Plasma NEFA (μeq/L)	151 ± 29	123 ± 33	259 ± 77	214 ± 111
Plasma IRI (μU/ml)	31 ± 6	22 ± 5	80 ± 35	38 ± 12*
16				
Body weight (g)	284 ± 11	279 ± 14	636 ± 43	585 ± 62
Blood glucose (mg/dl)	90 ± 5	86 ± 5	89 ± 7	83 ± 32
Plasma TG (mg/dl)	131 ± 15	129 ± 26	270 ± 146	85 ± 35*
Plasma NEFA (μeq/L)	205 ± 49	150 ± 69	231 ± 80	339 ± 340
Plasma IRI (μU/ml)	46 ± 20	54 ± 16	58 ± 21	31 ± 12*

ADD-3878 (100 mg/kg/day) was orally administered to young (5 wk old) and old Sprague-Dawley rats (20 wk old) for 16 days. Blood samples were taken 20 h after administration of ADD-3878.
N = 5. Mean ± SD. *P < 0.05, †P < 0.02, ‡P < 0.01, §P < 0.001 versus control.

and glucose intolerance. ADD-3878 was also effective on glucose and lipid metabolism in aged (old) rats, which were moderately insulin resistant and hyperlipidemic compared with young rats as reported elsewhere,[24,25] but not on the metabolism in young normal rats or streptozotocin-diabetic rats. Furthermore, in beagle dogs with mild intolerance to glucose, ADD-3878 ameliorated glucose tolerance and suppressed postprandial rises in plasma triglyceride and insulin. Therefore, ADD-3878 is more likely to be effective on the metabolism of carbohydrate and lipid in insulin-resistant or obese animals than in normal or insulin-deficient diabetic animals.

ADD-3878 markedly exaggerated glycemic response to injected insulin and also decreased the SSPG, which was in a reversed relationship to overall insulin sensitivity of the peripheral tissue[17] in the insulin-resistant animals. These results suggest that the primary action of ADD-3878 is to decrease insulin resistance of peripheral tissue. The above postulation concerning the mechanism of actions of ADD-3878 may be supported by our preliminary studies. ADD-3878 administration to yellow KK mice decreased insulin resistance of peripheral tissue manifested by increased insulin sensitivity and insulin responsiveness of adipocytes and soleus muscles (unpublished data).

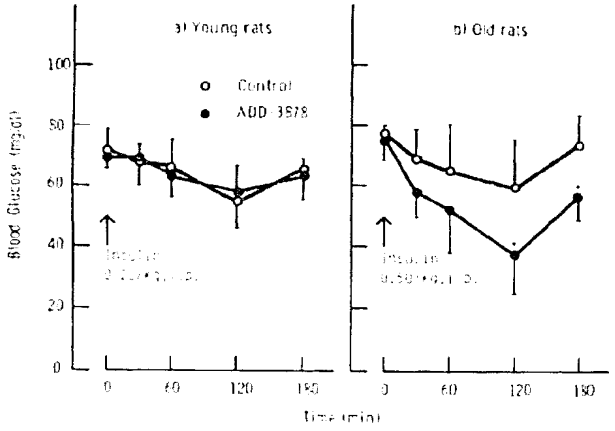

FIGURE 4. Effect of ADD-3878 on insulin tolerance in normal rats. Male Sprague-Dawley rats were used. ADD-3878 (100 mg/kg/day) was orally administered to young (6 wk old) and old (20 wk old) rats for 4 days. After the last administration, rats were fasted for 20 h and received an injection of insulin (0.2 or 0.5 U/kg, i.p.). *P < 0.05 versus control. Mean ± SD (N = 5).

TABLE 5
Effects of ADD-3878 on plasma glucose, insulin, triglyceride, and cholesterol in dogs

		ADD-3878 treatment		
		Before	After	(% initial)
Fasting plasma				
Glucose (mg/dl)		101 ± 4	92 ± 6	(92 ± 8)
IRI (μU/ml)		7.5 ± 1.8	7.6 ± 0.6	(105 ± 24)
Triglyceride (mg/dl)		25 ± 6	19 ± 7	(78 ± 27)
Cholesterol (mg/dl)		125 ± 26	93 ± 20	(74 ± 5†)
Postprandial plasma				
Glucose (mg/dl)	2 h	98 ± 3	96 ± 2	(98 ± 3)
	3 h	108 ± 6	100 ± 4	(93 ± 2†)
IRI (μU/ml)	2 h	14.6 ± 5.3	12.5 ± 6.1	(107 ± 85)
	3 h	24.8 ± 8.3	13.5 ± 2.7	(58 ± 21*)
Triglyceride (mg/dl)	2 h	76 ± 39	43 ± 16	(61 ± 17†)
	3 h	79 ± 40	51 ± 25	(72 ± 43)

ADD-3878 (100 mg/kg/day) was orally administered to male beagle dogs after meals for 4 days. Values are represented as means ± SD of five dogs.
Fasting plasma determinations were obtained before the last administration of ADD-3878.
*P < 0.02, †P < 0.01, ‡P < 0.001 versus initial.

Insulin sensitivity is decreased with the advance of age in normal and genetically obese animals as well as in human subjects.[9,10,26] The age-dependent elevation of plasma glucose, insulin, and triglyceride in yellow KK mice and fatty rats appears to be closely related to increasing peripheral insulin resistance. The chronic administration of ADD-3878 for as long as 12 wk suppressed the age-dependent rises of the three parameters in yellow KK mice and fatty rats. These results also suggest the inhibitory action of ADD-3878 on insulin resistance.

From the pathogenic point of hyperglycemia and hyperinsulinemia, the effects of ADD-3878 on plasma glucose and insulin are clearly explained. Insulin resistance is assumed to lead to a compensatory adaptation of pancreatic B-cells, which results in hyperinsulinemia, although the feedback mechanism between insulin-resistant tissue and pancreatic B-cells remains unclear. In regard to impaired glucose metabolism acompanied by hyperinsulinemia, insulin resistance and the pancreatic compensation are the main determinants of glucose intolerance and/or hyperglycemia. Based on these concepts, the reduction of insulin resistance by ADD-3878 probably results in normalization of hyperglycemia and hyperinsulinemia of the insulin resistant animal models.

Hyperinsulinemia and hyperlipemia are very common syndromes in obesity and diabetes. However, less information on the pathogenic action of insulin resistance has been presented for hyperlipemia than for hyperglycemia. Hypertriglyceridemia due to increased hepatic triglyceride output may be caused by hyperinsulinemia in the insulin-resistant state, because insulin is one of the potent stimulators of lipid synthesis and, furthermore, hepatic lipid synthesis is still sensitive to insulin in obese and diabetic animals and humans.[9,10,27] On the other hand, insulin resistance may decrease lipid uptake through decreasing insulin actions on lipoprotein lipase induction or glucose metabolism, which supplied substrates for esterification of fatty acids. The present studies clearly demonstrated that ADD-3878 administration to fatty rats increased the rate of lipid removal but did not affect hepatic triglyceride output. The insulin-resistance decreasing mechanism may be involved in the lipid-lowering action of ADD-3878.

ADD-3878 has neither acute hypoglycemic action nor stimulatory action on insulin secretion in the animals. In this regard, it can be distinguished from sulfonylureas. In other insulin-resistant mice such as the db/db mice, tolbutamide failed to decrease blood glucose[28] but ADD-3878 decreased both hyperglycemia and hyperinsulinemia (unpublished data). Furthermore, in our preliminary study, yellow KK mice did not show any response to tolbutamide (unpublished data). Although extrapancreatic actions of sulfonylureas have been proposed,[29-34] so far as examined in experimental animals, ADD-3878 is quite different from sulfonylureas in the effects on insulin-resistant or obese animals. It is postulated that ADD-3878 is more beneficial to obese and/or type II diabetes than sulfonylureas by its specific action, reduction of insulin resistance.

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